DEPARTMENT OF HEALTH & HUMAN SERVICES

Public Health Service

P920006

Memorandum

Date SEP 26 1995

From Director, Office of Device Evaluation (HFZ-400)
Center for Devices and Radiological Health (CDRH)

Subject Premarket Approval of Abbott Laboratories
Abbott PgR-ICA Monoclonal - ACTION

To The Director, CDRH
ORA _____

ISSUE. Publication of a notice announcing approval of the subject PMA.

FACTS. Tab A contains a FEDERAL REGISTER notice announcing:

(1) a premarket approval order for the above referenced medical device (Tab B); and

(2) the availability of a summary of safety and effectiveness data for the device (Tab C).

RECOMMENDATION. I recommend that the notice be signed and published.

Susan Alpert, Ph.D., M.D.

Attachments
Tab A - Notice
Tab B - Order
Tab C - S & E Summary

DECISION

Approved _____ Disapproved _____ Date _____

Prepared by C. Ann Hawthorne, CDRH, HFZ-440, August 9, 1995, 594-1243

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DRAFT

DEPARTMENT OF HEALTH AND HUMAN SERVICES FOOD AND DRUG ADMINISTRATION [DOCKET NO.

Abbott Laboratories; PREMARKET APPROVAL OF Abbott PGR-ICA Monoclonal

AGENCY: Food and Drug Administration, HHS.

ACTION: Notice.

SUMMARY: The Food and Drug Administration (FDA) is announcing its approval of the application by Abbott Laboratories, Abbott Park, IL, for premarket approval, under section 515 of the Federal Food, Drug, and Cosmetic Act (the act), of Abbott PGR-ICA Monoclonal.

DATE: Petitions for administrative review by (insert date 30 days after date of publication in the FEDERAL REGISTER).

ADDRESS: Written requests for copies of the summary of safety and effectiveness data and petitions for administrative review to the Dockets Management Branch (HFA-305), Food and Drug Administration, Rm. 1-23, 12420 Parklawn Drive, Rockville, MD 20857.

FOR FURTHER INFORMATION CONTACT:

Cornelia Rooks

Center for Devices and Radiological Health (HFZ-440)

Food and Drug Administration

9200 Corporate Blvd.
Rockville, MD 20850
301-594-1243.

SUPPLEMENTARY INFORMATION: On February 6, 1992, Abbott Laboratories, Abbott Park, IL 60064-3500, submitted to CDRH an application for premarket approval of Abbott PGR-ICA Monoclonal. The device is for the detection of human progesterone receptor (PgR) in breast tumor tissue to be used as an aid in assessing the likelihood of response to hormonal therapy, and as an aid in the prognosis and management of breast cancer patients. In accordance with the provisions of section 515(c)(2) of the act as amended by the Safe Medical Devices Act of 1990, this PMA was not referred to the Clinical Chemistry and Clinical Toxicology Devices Panel, an FDA advisory panel, for review and recommendation because the information in the PMA substantially duplicates information previously reviewed by this panel. On September 26, 1995, CDRH approved the application by a letter to the applicant from the Director of the Office of Device Evaluation, CDRH.

A summary of the safety and effectiveness data on which CDRH based its approval is on file in the Dockets Management Branch (address above) and is available from that office upon written request. Requests should be identified with the name of the device and the docket number found in brackets in the heading of this document.

OPPORTUNITY FOR ADMINISTRATIVE REVIEW

Section 515(d)(3) of the act (21 U.S.C. 360e(d)(3)) authorizes any interested person to petition, under section 515(g) of the act (21 U.S.C. 360e(g)), for administrative review of CDRH's decision to approve this application. A petitioner may request either a formal hearing under part 12 (21 CFR part 12) of FDA's administrative practices and regulations or a review of the application and CDRH's action by an independent advisory committee of experts. A petition is to be in the form of a petition for reconsideration under 10.33(b) (21 CFR 10.33(b)). A petitioner shall identify the form of review requested (hearing or independent advisory committee) and shall submit with the

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petition supporting data and information showing that there is a genuine and substantial issue of material fact for resolution through administrative review. After reviewing the petition, FDA will decide whether to grant or deny the petition and will publish a notice of its decision in the FEDERAL REGISTER. If FDA grants the petition, the notice will state the issue to be reviewed, the form of the review to be used, the persons who may participate in the review, the time and place where the review will occur, and other details.

Petitioners may, at any time on or before (insert date 30 days after date of publication in the FEDERAL REGISTER), file with the Dockets Management Branch (address above) two copies of each petition and supporting data and information, identified with the name of the device and the docket number found in brackets in the heading of this document. Received petitions may be seen in the office above between 9 a.m. and 4 p.m., Monday through Friday.

This notice is issued under the Federal Food, Drug, and Cosmetic Act (secs. 515(d), 520(h), and (21 U.S.C. 360e(d), 360j(h)) and under authority delegated to the Commissioner of Food and Drugs (21 CFR 5.10) and redelegated to the Director, Center for Devices and Radiological Health (21 CFR 5.53).

Dated:_____.

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DEPARTMENT OF HEALTH & HUMAN SERVICES — Public Health Service

Food and Drug Administration
2098 Gaither Road
Rockville MD 20850

Ms. Joy C. Sonsalla
Senior Regulatory Administrator
ADD Regulatory Affairs
Diagnostics Division
Abbott Laboratories
Abbott Park, IL 60064

SEP 26 1995

Re: P920006
Abbott PgR-ICA Monoclonal
Filed: February 6, 1992
Amended: February 26, April 20, May 1, and November 18, 1992; January 11, February 19, November 8, 1993, May 10, and November 4, 1994; and July 17, August 10, and August 21, 1995

Dear Ms. Sonsalla:

The Center for Devices and Radiological Health (CDRH) of the Food and Drug Administration (FDA) has completed its review of your premarket approval application (PMA) for the Abbott PgR-ICA Monoclonal. This device is indicated for the detection of human progesterone receptor (PgR) in breast tumor tissue to be used as an aid in assessing the likelihood of response to hormonal therapy, and as an aid in the prognosis and management of breast cancer patients. We are pleased to inform you that the PMA is approved subject to the conditions described below and in the "Conditions of Approval" (enclosed). You may begin commercial distribution of the device upon receipt of this letter.

The sale, distribution, and use of this device are restricted to prescription use in accordance with 21 CFR 801.109.

Expiration dating for this device has been established and approved at 12 months when shipped at ambient temperatures and stored at 2-8°C.

CDRH will publish a notice of its decision to approve your PMA in the FEDERAL REGISTER. The notice will state that a summary of the safety and effectiveness data upon which the approval is based is available to the public upon request. Within 30 days of publication of the notice of approval in the FEDERAL REGISTER, any interested person may seek review of this decision by requesting an opportunity for administrative review, either through a hearing or review by an independent advisory committee, under section 515(g) of the Federal Food, Drug, and Cosmetic Act (the act).

Failure to comply with the conditions of approval invalidates this approval order. Commercial distribution of a device that is not in compliance with these conditions is a violation of the act.

You are reminded that as soon as possible, and before commercial distribution of your device, that you must submit an amendment to this PMA submission with copies of all approved labeling in final printed form.

All required documents should be submitted in triplicate, unless otherwise specified, to the address below and should reference the above PMA number to facilitate processing.

PMA Document Mail Center (HFZ-401)
Center for Devices and Radiological Health
Food and Drug Administration
9200 Corporate Blvd.
Rockville, Maryland 20850

If you have any questions concerning this approval order, please contact Cornelia Rooks or Ann Hawthorne (301) 594-1243.

Sincerely yours,

Susan Alpert, Ph.D., M.D.
Director
Office of Device Evaluation
Center for Devices and
Radiological Health

Summary of Safety and Effectiveness

I. General Information

Device Generic Name: Immunocytochemical assay for the Detection of Human Progesterone Receptor in Breast Tumor Tissue.

Device Trade Name: Abbott PgR-ICA Monoclonal

Applicant's Name and Address: Abbott Laboratories
Diagnostics Division
Abbott Park, IL
60064-3500

Premarket Approval Application: (PMA) P920006:

Panel Recommendations: Pursuant to section 515(c) of the act as amended by the Safe Medical Devices Act of 1990, this PMA was not the subject of an FDA Clinical Chemistry and Clinical Toxicology Devices Advisory Panel meeting because the information in the PMA substantially duplicates information previously reviewed by this panel.

Date of Notice of Approval of the Applicant: SEP 26 1995

II. Indications for Use

The Abbott PgR-ICA Monoclonal is an in vitro device for the detection of human progesterone receptor (PgR) in breast tumor tissue to be used as an aid in assessing the likelihood of response to hormonal therapy, and as an aid in the prognosis and management of breast cancer patients.

Background

Steroid hormone receptors have been shown to be important in the clinical management of breast cancer patients. In 1985, an NIH Consensus Committee and the American Cancer Society both published reports supporting the significance of steroid hormone receptor status in the management of breast cancer.[1,2]

The synthesis of progesterone receptor (PgR) in a tissue is dependent upon the action of estrogen. The presence of estrogen receptor (ER) or PgR within a tissue is generally considered a prerequisite for a

tissue to recognize estrogens, progestins or their analogs and to manifest long-term response to hormonal therapy.[3-5]

Terenius[6] was the first to report the presence of progestogen receptors in cytosols from human breast tumor tissue using [^{3}H]-progesterone. Horwitz and McGuire[7] confirmed these findings utilizing [^{3}H]-R5020 (Promegestone), a synthetic progestogen, to measure PgR binding. Subsequent studies by other investigators supported these findings.[8,9]

PgRs in tissue cytosol have been measured in vitro by sucrose density gradient (SDG) or dextran-coated charcoal (DCC) separation assays. Production of monoclonal antibodies against human progesterone receptor protein has permitted the development of assays based on direct antigenic recognition rather than steroid binding activity.[10-12] These assays measure PgR molecules whether or not they are bound to endogenous progesterone.[10] The antibodies have been shown by immunoblot analyses to recognize the 120K and the 95K molecular weight forms of human progesterone receptor.[10,11] Immunocytochemical assays demonstrate anti-PgR monoclonal antibody reactivity with target tissues and lack of reactivity with non-target tissues. These monoclonal antibodies also react with progesterone receptors in meningiomas.[10]

The receptor status of breast tumors is useful in predicting patient response to endocrine therapy. An analysis of 850 patients from 14 different studies has shown that approximately 53 percent of women with breast tumors containing ER respond objectively to endocrine therapy, and approximately 68 percent of women with breast tumors containing PgR respond objectively to endocrine therapy.[13]

The receptor status of primary breast cancer lesions is also useful in assessing patient prognosis following mastectomy. Patients with ER or PgR positive tumors have a longer disease-free interval and overall survival than do patients with receptor negative tumors.[14-16]

III. Device Description

The Abbott PgR-ICA Monoclonal system, hereafter referred to as PgR-ICA, employs a peroxidase-anti-peroxidase (PAP) technique for

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visualization of PgR in tissue specimens such as frozen tissue sections through the use of monoclonal antibodies directed specifically against the receptors.

Two samples from the same specimen are placed on separate microscope slides which have been treated with Tissue Adhesive. The samples are fixed in formaldehyde, methanol, and acetone and treated with the Blocking Reagent to prevent nonspecific binding of subsequent reagents. One of the two samples is incubated with the Primary Antibody, an IgG fraction of a monoclonal (rat) antibody to human PgR, and the other is incubated with the Control Antibody, normal rat IgG. The purpose of the Control Antibody is to evaluate nonspecific binding of the reagents to the sample. The slides are then incubated with the Bridging Antibody, a goat anti-rat IgG, which binds to rat antibody against human PgR in the sample treated with Primary Antibody and to normal rat IgG bound to the sample treated with Control Antibody. The rat PAP Complex is added to each sample and binds to the anti-rat IgG Bridging Antibody. A Chromogen-Substrate Solution containing Substrate Reagent (hydrogen peroxide) and DAB (diaminobenzidine·4 HCl) is added to each sample. The reaction of peroxidase with hydrogen peroxide converts the DAB to an insoluble reddish-brown product. Progesterone receptor-monoclonal antibody complexes can be visualized with a light microscope because of the amplification from the Bridging Antibody, PAP Complex, and enzyme reaction products.

Assay validity is determined by staining a positive control such as a frozen section of breast cancer tissue known to be PgR positive or the Abbott PgR-ICA Positive Control Slide.

IV. Alternative Practices and Procedures

Alternative practices and/or procedures for aiding in the management of breast cancer patients include other in vitro hormone receptor assays for which there are FDA approved PMAs. Other characteristics which have been shown to aid in determining patient prognosis include: histological status of axillary lymph nodes, primary tumor size, menopausal status or age, and tumor histopathology.[1]

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V. Marketing History

PgR-ICA has been marketed in Canada and West Germany since June, 1989 and Japan since 1990.

Abbott PgR-ICA Monoclonal has never been withdrawn from the market for any reason related to the safety or effectiveness of the device.

VI. Adverse Effects of the Device on Health

When this in vitro device is used as indicated, it is possible that:

A false-negative result could lead to a medical decision depriving the patient of potentially beneficial endocrine therapy.

A false-positive result could lead to medical decisions causing a patient to undergo inappropriate therapy.

There are no other known potential adverse effects in the health of the patient if this in vitro device is used according to the instructions.

VII. Summary of Studies

A. Nonclinical studies

Preclinical laboratory studies were conducted to determine the purity and specificity of the reagents as well as assay performance.

1. Purity and Identity of the Antigen

Nuclear extract PgR was purified from a human breast cancer cell line. The PgR was characterized by its sedimentation profile when bound to [^{3}H]-ORG.2058 in low and high salt sucrose gradients.

In addition, the partially purified and immunopurified PgR were also analyzed by sodium dodecyl sulfate-polyacrylamide gel electrophoresis and the results agreed with published molecular weight values. These preparations were judged suitable for use as immunogens.

2. Specificity of the Antibody

Studies by Abbott Laboratories and the University of Chicago showed that the anti-PgR monoclonal antibody quantitatively shifted the sedimentation profile of [^{3}H]-ORG.2058 labeled PgR from 4S to 7-8S. A double antibody assay demonstrated antibody binding to PgR but a lack of binding to estrogen, androgen or glucocorticoid receptor.

The resulting sedimentation profile showed a shift characteristic of an antibody-PgR-^{3}H-ORG.2058 complex. Of the receptors tested, the antibody only recognized progesterone receptor.

3. Performance Characteristics

a. Reproducibility

Reproducibility was evaluated by three technicians who performed four assay runs (replicates of three) with one lot of the PgR-ICA Monoclonal on three positive breast tumor specimens and one negative tumor specimen. The number and percent of PgR positive tissue sections were determined for each tumor. Within run percent coefficients of variation, (CVs) were calculated for replicates of three. Between run and between technician percent CVs were projected for replicates of one. PgR positive is defined as equal to or more than 10 percent cells stained (CS). PgR negative is defined as less than 10 percent CS. The results of these studies are shown in Table 1. The within run, between run and between technician percent CVs for the three positive specimens ranged from 2.8 to 6.7, 3.2 to 17.4, and 3.2 to 17.6, respectively. All sections from the negative tumor were negative and all sections from all positive tumors were positive.

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Table 1

Within Lot Reproducibility of PgR-ICA

Tumor	% Positive	Mean Percent Cells Stained	Within Run % CV	Between Run % CV	Between Technician % CV
1 A	0(0/36)	0	0.0	0.0	0.0
1 B	100(36/36)	66	6.7	17.4	17.6
1 C	100(36/36)	81	4.1	6.4	6.6
1 D	100(36/36)	91	2.8	3.2	3.2

To determine lot-to-lot reproducibility, 19 tumor specimens were assayed using three different lots of PgR-ICA. The values obtained for these specimens are presented in Table 2. The tumor sections were 100 percent positive at 20-85 percent CS and 100 percent negative at 0-3 percent CS. At 10 percent CS, two sections were positive (11 percent CS) and one was negative (7 percent CS).

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Table 2

Between Lot Reproducibility of PqR-ICA

Percent Cells Stained

Tumor	% Positive	Lot 1	Lot 2	Lot 3	Mean	Between Lot % CV
2 A	100(3/3)	86	85	83	85	1.8
2 B	100(3/3)	85	76	82	81	5.7
2 C	100(3/3)	78	77	73	76	3.4
2 D	100(3/3)	73	75	73	74	1.6
2 E	100(3/3)	58	55	51	55	6.4
2 F	100(3/3)	56	63	54	58	8.1
2 G	100(3/3)	50	55	47	51	8.0
2 H	100(3/3)	49	51	47	49	4.1
2 I	100(3/3)	48	44	46	46	4.4
2 J	100(3/3)	44	40	39	41	6.3
2 K	100(3/3)	44	45	47	45	3.3
2 L	100(3/3)	30	37	31	33	11.5
2 M	100(3/3)	28	35	36	33	13.3
2 N	100(3/3)	26	23	18	22	18.2
2 O	100(3/3)	23	19	19	20	11.5
2 P	67(2/3)	11	11	7	10	23.0
2 Q	0(0/3)	3	3	4	3	20.0
2 R	0(0/3)	0	0	0	0	0.0
2 S	0(0/3)	0	0	0	0	0.0

To determine specimen preparation reproducibility, 7 tumor specimens were sectioned, fixed and assayed in duplicate by four technicians and evaluated by one observer (Table 3). Between technician percent CVs ranged from 6.4 to 16.5. The tumor sections were 100 percent negative at 6 percent CS and 100 percent positive at 40-74 percent CS for one observer.

Table 3

Specimen Preparation Reproducibility of PgR-ICA

Tumor	% Positive	Mean Percent Cells Stained	Within Run % CV	Between Tech % CV
3A	0(0/8)	6	7.4	11.9
3B	100(8/8)	40	10.8	13.8
3C	100(8/8)	44	4.1	9.0
3D	100(7/7)*	54	11.7	16.5
3E	100(8/8)	66	8.9	8.9
3F	100(7/7)*	72	2.5	6.4
3G	100(7/7)*	74	4.0	7.7

*Section lost due to technician error.

To determine inter-observer reproducibility, 9 PgR positive tumor specimens were sectioned, fixed and assayed in duplicate by one technician and evaluated by three independent observers. The values obtained for these specimens are presented in Table 4. Between observer percent CVs ranged from 3.9 to 27.8. The tumor sections were 100 percent positive at 46-89 percent CS.

Table 4

Inter-Observer Reproducibility of PgR-ICA

Tumor	% Positive	Mean Percent Cells Stained	Within Observer % CV	Between Observer % CV
4 A	100(6/6)	46	12.8	14.3
4 B	100(6/6)	66	6.6	27.8
4 C	100(6/6)	62	4.2	21.9
4 D	100(6/6)	71	8.0	19.9
4 E	100(6/6)	74	3.9	16.8
4 F	100(6/6)	80	3.0	5.6
4 G	100(6/6)	82	5.6	5.6
4 H	100(6/6)	85	4.0	3.9
4 I	100(6/6)	89	3.0	5.6

b. Stability

The stability of PgR-ICA was determined by assaying the PgR-ICA

Control slide and a PgR-ICA positive breast tumor slide with different reagent lots subjected to the following conditions.

Storage Condition

1. 2 to 8°C continuous

2. Room temperature continuous

3. 45°C for 3 days then 2 to 8°C continuous

Results of the stability studies support 12 month expiration dating for the PgR-ICA.

c. Specificity

Studies were conducted to assess the specificity of PgR-ICA.

Comparisons of breast tumor tissue stained by Primary and Control Antibody demonstrated the absence of non-specific staining. Cell lines with PgR content known by DCC separation assays were assayed by the PgR-ICA. No staining by the PgR-ICA was observed in all lines defined as PgR negative by DCC. Progesterone receptor staining was observed in all cell lines defined as PgR positive by DCC. Progesterone target and non-target human tissues were assayed by the PgR-ICA. Target tissues exhibited positive staining, whereas non-target tissues did not. Other studies supporting the specificity of this PgR-ICA primary antibody have been published[10,17].

d. Interfering Substances

Tumor sections were incubated with potentially interfering substances prior to performing PgR-ICA

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Monoclonal. Aminoglutethimide, androstan, diethylstilbestrol, dexamethasone, estradiol, megestrol acetate, prednisone, progesterone, 4-hydroxy-tamoxifen and tamoxifen were tested at concentrations of 10^{-7} and 10^{-9} M. Lidocaine was tested at 100 and 10 mg/mL. No interference was observed.

e. Reproducibility

Reproducibility was evaluated at the four clinical sites. Within run (intra-assay), between run (inter-assay) and between lab reproducibilities were evaluated by performing four assay runs (replicates of three) of the PgR-ICA Monoclonal on three positive breast tumor specimens and one negative tumor specimen. The results of these studies are shown in Table 5.

Table 5

Reproducibility of PgR-ICA

Tumor	% Positive	Mean Percent Cells Stained	Within Run % CV	Between Run % CV	Between Lab % CV
A	0(0/47)*	1	NA	NA	NA
B	100(48/48)	44	18.0	21.2	53.1
C	100(48/48)	87	3.3	5.6	16.0
D	100(48/48)	93	2.0	3.0	7.4

*One slide at Site II was not evaluated.
NA = Not applicable.

The 53.1 percent CV observed for Tumor B resulted directly from its being an heterogenous and atypical breast cancer tumor. Using a cutoff of 10 percent CS, all investigators found Tumor B to be positive. Within run percent CVs on Tumors C and D were 3.3 and 2.0, between run percent CVs were 5.6 and 3.0, and between lab percent

CVs were 16.0 and 7.4, respectively. The mean result for the negative tumor sections was 1 percent CS. The mean percent CS for each positive tumor ranged between 44 and 93 percent CS.

To determine inter-observer reproducibility, 11 pre-stained tumor sections from 11 cancer tumors were evaluated by an observer within Abbott and a certified pathologist. The values obtained for these specimens are presented in Table 6.

Table 6

Inter-Observer Reproducibility of PgR-ICA

	% Cells Stained		
Tumor	Observer 1	Observer 2	Difference
1	45	25	20
2	36	30	6
3	52	50	2
4	62	60	2
5	71	70	1
6	79	70	9
7	92	90	2
8	18	18	0
9	0	0	0
10	10	7	3
11	3	7	-4
		Mean Difference = 3.73	

Agreement between the two observers was achieved (r=0.9801) and is comparable to the inter-observer reproducibility reported in Table 4. For all but one specimen, the values for percent cells stained were within 10 percent CS of each other. Using a cutoff of 10 percent CS, 100 percent concordance was achieved between observers.

B. Clinical Studies

Four investigators conducted retrospective studies on clinical samples to evaluate

PgR-ICA. The investigators and study periods were: Site I: Louis P. Pertschuk, D.O., State University of New York Downstate Medical Center, Brooklyn (December 1988 - May 1990); Site II: Lambert Skoog, M.D., Ph.D., Karolinska Hospital, Stockholm (February 1989 - May 1990); Site III: R. Charles Coombes, M.D., Ph.D., F.R.C.P., St. George's Hospital Medical Center, London (December 1988 - May 1990); and Site IV: Prof. Dr. med. Wolfgang Remmele, Klinikum der Landeshauptstadt Wiesbaden (January 1989 - May 1990). Specimens included in the utility studies were selected on the basis of the availability of adequate clinical information. The percentage of stained tumor cells was determined for each specimen assayed by PgR-ICA.

Eight different master lots of the PgR-ICA were used in the clinical studies. The number of master lots used by each site was as follows:

Site	Number of Lots
I	5
II	4
III	2
IV	1

A total of 106 breast tumor specimens from Sites I, II and III were evaluable for correlating the PgR-ICA result to patient response to hormonal therapy for advanced breast cancer. Patient response to hormonal therapy was assessed by objective criteria and categorized by patient response as defined by the International Union of Clinical Chemists, (UICC).[18] When a cutoff value of 10 percent cells stained was applied to these data, sensitivity and specificity were 69 percent and 74 percent, respectively (Table 7). Fifty-one percent (27/53) of PgR-ICA positive patients responded to hormonal therapy. Among the PgR-ICA positive patients (40/53) 75 percent responded to or were stable on hormonal therapy. Seventy percent (37/53) of PgR-ICA negative patients developed progressive disease on hormonal therapy.

Table 7

Clinical Agreement Between PgR-ICA Measurements and Response to Hormonal Therapy

	Responders	Non-Responders	No Change	Total
PgR-ICA (+)	27	13	13	53
PgR-ICA (-)	12	37	4	53

Sensitivity = 69% (27/39)
Specificity = 74% (37/50)

A total of 325 primary breast tumor specimens from Sites I, II and III were evaluable for a comparison of PgR-ICA result to overall patient survival (mastectomy to death or date of last physician contact). A cutoff of 10 percent CS was used to distinguish PgR-ICA positive from PgR-ICA negative results. The overall survival of patients with PgR-ICA positive tumors was statistically significantly longer, 96 percent at 36 months, 90 percent at 60 months, than that observed for patients with PgR-ICA negative tumors, 70 percent at 36 months, 47 percent at 60 months ($p<0.0001$). When PgR-ICA results were stratified by stage, menopausal status and pathologic nodal status, PgR positive patients demonstrated statistically significantly longer overall survival than PgR negative patients ($p<0.05$). Survival rates for PgR positive patients at 36 months were 100 percent for Stage I, 94 percent for Stage II, 100 percent for Stage III, 96 percent for pre/perimenopausal, 96 percent for postmenopausal, 98 percent for 0-3 positive nodes and 92 percent for greater than 3 positive nodes. Survival rates for PgR negative patients at 36 months were 86 percent for Stage I, 70 percent for Stage II, 57 percent for Stage III, 72 percent for pre/perimenopausal, 70 percent for postmenopausal, 78 percent for 0-3 positive nodes and 60 percent for greater than 3 positive nodes. Survival rates for PgR positive patients at 60 months were 95

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percent for Stage I, 87 percent for Stage II, 94 percent for Stage III, 84 percent for pre/perimenopausal, 92 percent for postmenopausal, 94 percent for 0-3 positive nodes and 82 percent for greater than 3 positive nodes. Survival rates for PgR negative patients at 60 months were 67 percent for Stage I, 50 percent for Stage II, 19 percent for Stage III, 48 percent for pre/perimenopausal, 48 percent for postmenopausal, 64 percent for 0-3 positive nodes and 31 percent for greater than 3 positive nodes. These data are shown in Table 8.

Table 8

Overall Survival Rates

	36 Months		60 Months	
	PgR-ICA(+)	PgR-ICA(-)	PgR-ICA(+)	PgR-ICA(-)
Total Population	96%	70%	90%	47%
Stage I	100%	86%	95%	67%
Stage II	94%	70%	87%	50%
Stage III	100%	57%	94%	19%
Pre/Perimenopausal	96%	72%	84%	48%
Postmenopausal	96%	70%	92%	48%
0-3 Positive Nodes	98%	78%	94%	64%
>3 Positive Nodes	92%	60%	82%	31%

A total of 322 primary breast tumor specimens from Sites I, II and III were evaluable for a comparison of PgR-ICA result to patient disease-free interval (mastectomy to recurrence or, if no recurrence, date of last physician contact). A cutoff of 10 percent CS was used to distinguish PgR-ICA positive from PgR-ICA negative results. The disease-free interval of patients with PgR-ICA positive tumors was significantly longer, 75 percent at 36 months, 64 percent at 60 months than that observed for patients with PgR-ICA negative tumors 40 percent at 36 months, 27 percent at 60 months, ($p<0.0001$). When PgR-ICA results were stratified by stage, menopausal status and pathologic nodal status, PgR positive patients demonstrated statistically significantly

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longer disease-free interval than PgR negative patients ($p<0.05$). Disease-free interval rates at 36 and 60 months are shown in Table 9.

Table 9

Disease-Free Interval Rates

	36 Months		60 Months	
	PgR-ICA(+)	PgR-ICA(-)	PgR-ICA(+)	PgR-ICA(-)
Total Population	75%	40%	64%	27%
Stage I	95%	51%	77%	38%
Stage II	77%	40%	67%	29%
Stage III	40%	30%	30%	0%
Pre/Perimenopausal	76%	38%	65%	20%
Postmenopausal	74%	40%	63%	28%
0-3 Positive Nodes	80%	50%	71%	39%
>3 Positive Nodes	68%	29%	50%	14%

VIII. Conclusions Drawn from the Studies

Based upon the results obtained; the Abbott PgR-ICA Monoclonal has been demonstrated to be reasonably safe and effective for its intended use. The specificity of the monoclonal antibody was demonstrated by sucrose density gradient analysis as well as by interference studies using other steroid receptors. The assay detects and localizes PgR with a specificity of 100 percent as evidenced by studies of cell lines whose receptor content was known and by studies of progesterone target and non-target human tissue. The within run, between run, between technician, between lot, sample preparation and inter-observer reproducibilities were within acceptable ranges for immunocytochemical assays for all typical tumor sections studied.

Clinical studies demonstrated that the PgR-ICA is clinically useful as an aid in assessing the likelihood of response to hormonal therapy. One hundred six breast tumor specimens were evaluated for this study. With a cutoff of 10 percent CS, the sensitivity and specificity of the PgR-ICA as compared to clinical response, is 69 percent and 74 percent, respectively.

Clinical studies also demonstrated that the

PgR-ICA is clinically useful as an aid in assessing prognosis and management of breast cancer patients. The overall survival and disease-free interval of patients (n = 325 and 322, respectively) with PgR-ICA positive primary tumors was statistically significantly longer than that of patients with PgR-ICA negative tumors ($p<0.0001$).

These data support the clinical utility of PgR-ICA for the detection of human PgR in breast tumor tissue to be used as an aid in assessing the likelihood of response to hormonal therapy, and as an aid in the prognosis and management of breast cancer patients.

IX. Panel Recommendation

Pursuant to section 515(c) of the act as amended by the Safe Medical Devices Act of 1990, this PMA was not the subject of an FDA Clinical Chemistry and Clinical Toxicology Devices Advisory Panel meeting because the information in the PMA substantially duplicates information previously reviewed by this panel.

X. CDRH Action on the Application

An approval letter was issued to Abbott Laboratories on September 26, 1995.

The applicant's manufacturing and control facilities were inspected on February 17, 1993 for compliance with the Device Good Manufacturing Practice Regulations (GMPs), and the facilities were found to be in compliance.

The shelf life of the PgR-ICA has been established at 12 months when shipped at ambient temperatures and stored at 2-8°C.

XI. Approval Specifications

Directions for use: See attached labeling (Attachment A).

Conditions of Approval: CDRH approval of this PMA is subject to full compliance with the conditions described in the approval order (Attachment B).

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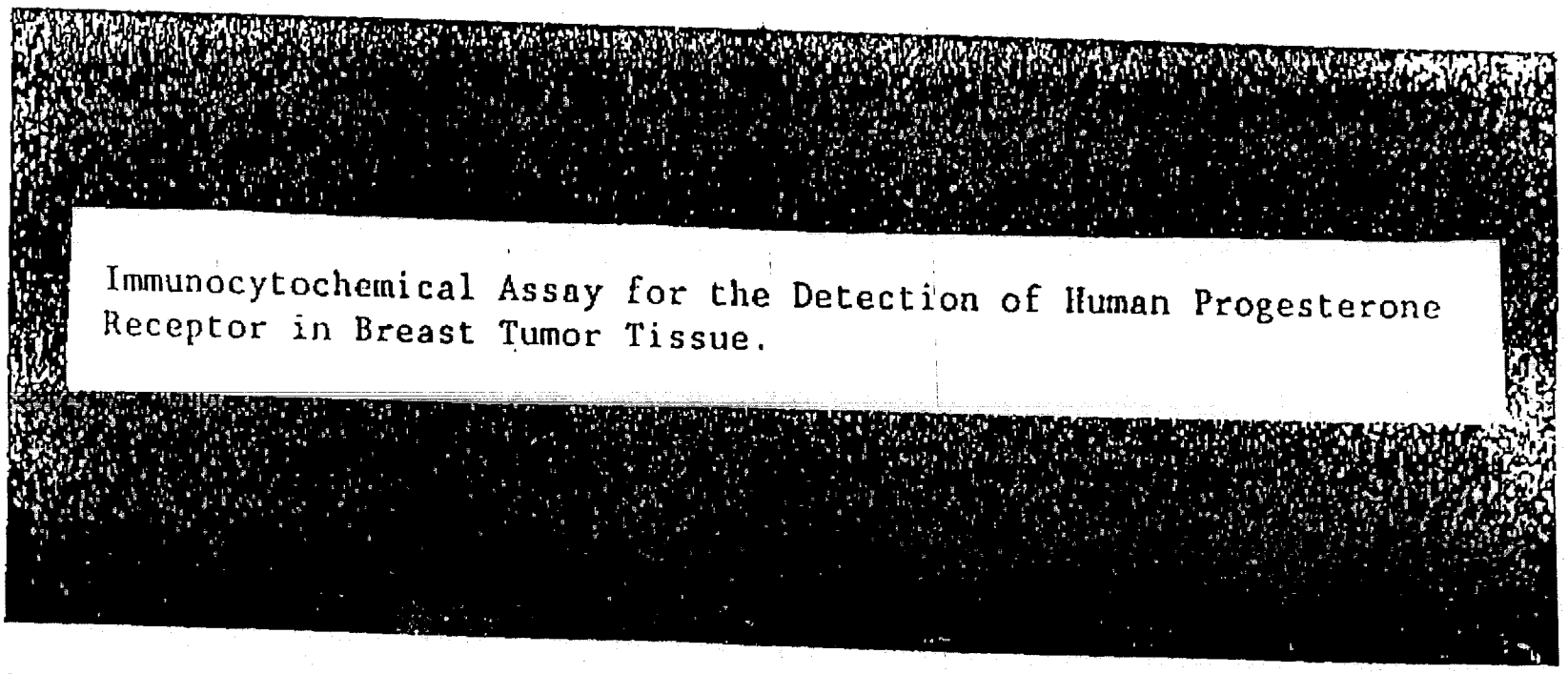
ABBOTT PgR-ICA Monoclonal

ABBOTT LABORATORIES
Diagnostics Division



CUSTOMER SUPPORT CENTER (USA)
1-800/323-9100

CAUTION: United States Federal law restricts this device to sale and distribution by or on the order of a physician, or to a clinical laboratory; and use is restricted to by or on the order of a physician.

NAME AND INTENDED USE

The Abbott PgR-ICA Monoclonal is an immunocytochemical assay for the detection of human progesterone receptor (PgR) in breast tumor tissue to be used as an aid in assessing the likelihood of response to hormonal therapy, and as an aid in the prognosis and management of breast cancer patients.

[WARNING]

This assay should be interpreted by a specialist in breast cancer morphology and/or pathology. Assay results should be used in conjunction with other clinical and laboratory data to assist the clinician in making individualized patient management decisions.

[PRECAUTIONS]
INSERT A

[DESCRIPTION OF THE PROCEDURE]

INSERT B

SUMMARY AND EXPLANATION OF TEST

Steroid hormone receptors have been shown to be important in the clinical management of breast cancer patients. In 1985, an NIH Consensus Committee and the American Cancer Society both published reports supporting the significance of steroid hormone receptor status in the management of breast cancer.[1,2]

The synthesis of progesterone receptor (PgR) in a tissue is dependent upon the action of estrogen. The presence of estrogen receptor (ER) or PgR within a tissue is generally considered a prerequisite for a tissue to recognize estrogens, progestins or their analogs and to manifest long-term response to hormonal therapy.[3-5]

Terenius[6] was the first to report the presence of progestogen receptors in cytosols from human breast tumor tissue using [^{3}H]-progesterone. Horwitz and McGuire[7] confirmed these findings utilizing [^{3}H]-R5020 (Promegestone), a synthetic progestogen, to measure PgR binding. Subsequent studies by other investigators supported these findings.[8,9]

Progestogen receptors in tissue cytosol have been measured in-vitro by sucrose density gradient (SDG) or dextran-coated charcoal (DCC) separation assays. Production of monoclonal antibodies against human progesterone receptor protein* has permitted the development of assays based on direct antigenic recognition rather than steroid binding activity.[10-12] These assays measure PgR molecules whether or not they are bound to endogenous progesterone.[10] The antibodies have been shown by immunoblot analyses to recognize the 120K and the 95K molecular weight forms of human progesterone receptor.[10,11] Immunocytochemical assays demonstrate anti-PgR monoclonal antibody reactivity with target tissues and lack of reactivity with non-target tissues. These antibodies also react with progesterone receptors in meningiomas.[10]

The receptor status of breast tumors is useful in predicting patient response to endocrine therapy. An analysis of 850 patients from 14 different studies has shown that approximately 53% of women with breast tumors containing ER respond objectively to endocrine therapy, and approximately 68% of women with breast tumors containing PgR respond objectively to endocrine therapy.[13]

The receptor status of primary breast cancer lesions is also useful in assessing patient prognosis following mastectomy. Patients with ER or PgR positive tumors have a longer disease-free interval and overall survival than do patients with receptor negative tumors. [13-14] 13-15

*U.S. Patent 4,742,000.

Insert A
Benign epithelial cells may contain progesterone receptor and may, therefore, stain positively with the PgR-ICA assay.

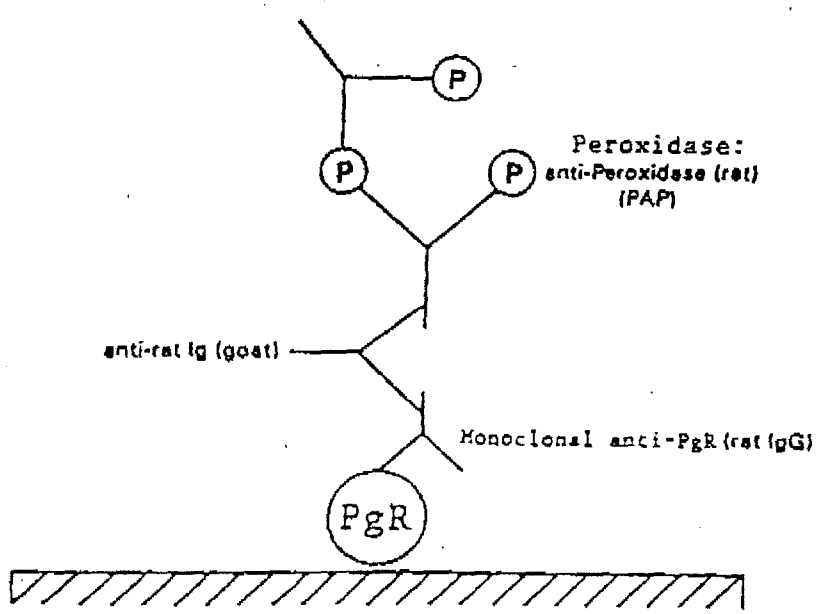
DESCRIPTON

~~BIOLOGICAL PRINCIPLE~~ OF THE PROCEDURE

The ABBOTT PgR-ICA Monoclonal system employs a ~~sensitive~~ peroxidase-anti-peroxidase technique for visualization of progesterone receptor in specimens such as frozen tissue sections through the use of a monoclonal antibody directed specifically against the receptor.[10] (PAP)

Two samples from the same specimen are placed on separate microscope slides which have been treated with Tissue Adhesive. The samples are fixed in formaldehyde, methanol, and acetone and treated with the Blocking Reagent to prevent nonspecific binding of subsequent reagents. One of the two samples is incubated with the Primary Antibody, an IgG fraction of a monoclonal (rat) antibody to human PgR, and the other is incubated with the Control Antibody, normal rat IgG. The purpose of the Control Antibody is to evaluate nonspecific binding of the reagents to the sample. The slides are then incubated with the Bridging Antibody, a goat anti-rat IgG which binds to rat antibody against human PgR in the sample treated with the Primary Antibody and to normal rat IgG bound to the sample treated with Control Antibody. The rat PAP Complex is added to each sample and binds to the anti-rat IgG Bridging Antibody. A Chromogen-Substrate Solution containing Substrate Reagent (hydrogen peroxide) and DAB (diaminobenzidine • 4 HCl) is added to each sample. The reaction of peroxidase with hydrogen peroxide converts the DAB to an insoluble reddish-brown product. Progesterone receptor-monoclonal antibody complexes can be visualized with a light microscope because of the amplification from the Bridging Antibody, PAP Complex, and enzyme reaction products.

Assay validity is determined by staining a positive control such as a frozen section of breast cancer tissue known to be PgR positive or the Abbott PgR-ICA Control Slide (List 2A91).



REAGENTS

ABBOTT PgR-ICA Monoclonal Kit, 30 Test Units

① 1 Vial (6 mL) Blocking Reagent. Normal Goat Serum in Phosphate Buffer with Antimicrobial Agent.
② 1 Vial (3 mL) Primary Antibody. Anti-PgR (Rat, Monoclonal) Antibody. Minimum Concentration: 0.1 µg/mL in Phosphate Buffer with Protein Stabilizer and Antimicrobial Agent.
③ 1 Vial (6 mL) Bridging Antibody. Goat Anti-Rat Antibody. Minimum Concentration: 1.0 µg/mL in Phosphate Buffer with Antimicrobial Agent.
④ 1 Vial (6 mL) PAP Complex. Peroxidase (Horseradish): Anti-peroxidase (Rat) Complex. Minimum Concentration: 0.1 µg/mL in Tris Buffer with Protein Stabilizer and Antimicrobial Agent.
⑤ 1 Vial (15 tablets) DAB Tablets. Diaminobenzidine • 4 HCl.
⑥ 2 Bottles (55 mL each) Substrate Reagent. Citrate-Phosphate Buffer containing Hydrogen Peroxide and Antimicrobial Agents.
⑦ 1 Vial (3 mL) Control Antibody. Normal Rat Antibody. Minimum Concentration: 0.1 µg/mL in Phosphate Buffer with Protein Stabilizer and Antimicrobial Agent.
⑧ 1 Vial (3 mL) Tissue Adhesive. Protein Solution with Antimicrobial Agent.

INSTRUCTIONS FOR USE

~~WARNINGS AND PRECAUTIONS~~

FOR IN VITRO DIAGNOSTIC USE.

~~General Precautions~~

1. Benign epithelial cells may contain progesterone receptor and may, ~~therefore~~ stain positively with the PgR-ICA assay.
2. Abbott PgR-~~ICA Monoclonal~~ results should be interpreted by a specialist in breast cancer morphology and/or pathology.

Safety Precautions

1. Do not pipette by mouth.
2. Do not smoke, eat, or drink in areas in which specimens or kit reagents are handled.
3. Wear disposable gloves while handling kit reagents or specimens and wash hands thoroughly afterward.
4. Avoid splashing or forming an aerosol.
5. Decontaminate and dispose of specimens and all potentially contaminated materials as if they contain infectious agents. Solid wastes should either be incinerated or autoclaved at 121°C for an appropriate period of time. Due to variations among autoclaves and in waste configuration, each user must verify the effectiveness of their decontamination cycle using biological indicators. Liquid waste not containing acid and neutralized waste may be mixed with sodium hypochlorite in volumes such that the final mixture contains 1% sodium hypochlorite. Allow 30 minutes for effective decontamination.

   CAUTION: Liquid waste containing acid must be neutralized prior to the addition of sodium hypochlorite.
6. Spills should be wiped up thoroughly using an appropriate intermediate-to-high level disinfectant such as 1% sodium hypochlorite. Acid containing spills should be wiped dry and then disinfected. Materials used to wipe up spills should be added to biohazardous waste.
7. Use DAB in a fume hood. Wear gloves when handling DAB. If DAB comes into contact with skin, wash immediately with water.

Handling Precautions

1. Do not use kit components beyond the expiration date.
2. Do not mix reagents from different master lots.
3. Avoid microbial contamination of reagents
4. Do not expose Chromogen-Substrate Solution to strong light. Prevent contact of the Chromogen-Substrate Solution with any oxidizing agents.
5. Use frozen or fresh specimens for this procedure. Routinely fixed paraffin-embedded specimens may produce inconsistent results.
6. The use of other fixatives, reagent dilutions, incubation temperatures and times, rinse procedures, or counterstains can adversely affect results; therefore, the assay procedure must be strictly followed.
7. Do not attempt to block endogenous peroxidase activity.
8. Specimens must be handled properly to minimize loss of PgR (see *Specimen Collection and Preparation for Analysis*). Delay in freezing, or storage at temperatures warmer than −70°C may result in a loss of PgR.
9. Do not allow the specimen to dry at any time. Once mounted on a slide, the specimen must be covered with Specimen Storage Medium, PBS or reagent. The fixed specimen may be kept for a maximum of 2 hours in PBS bath until the *Immunocytochemical Staining Procedure* has begun or stored at −10° to −20°C in Specimen Storage Medium. Once the *Immunocytochemical Staining Procedure* has begun each step must be performed according to the time indicated.

INSTRUCTIONS FOR PREPARATION OF MICROSCOPE SLIDES

Slides may be treated with Tissue Adhesive up to one month before use with tissue sections. Use with other specimens such as intact cells may require freshly prepared slides.

1. Add 3 mL of Tissue Adhesive to 300 mL of distilled or deionized water in a staining jar. Mix thoroughly by stirring.
2. Place clean microscope slides in a slide rack and immerse in the Tissue Adhesive Solution for 5 minutes.
3. Rinse the treated slides in a distilled or deionized water bath for 1 minute. Repeat this step one time using fresh distilled or deionized water.
4. Air dry the slides at room temperature.
5. Store the slides in a clean slide container.

   NOTE: The Tissue Adhesive Solution prepared in Step 1 may be stored at 2 to 8°C and reused for up to 1 month.

INSTRUCTIONS FOR PREPARATION OF CHROMOGEN-SUBSTRATE SOLUTION

CAUTION: Allow DAB Tablets and Substrate Reagent to come to room temperature before opening.

Ten to fifteen minutes prior to Color Development, prepare the Chromogen-Substrate Solution by dissolving the DAB Tablet in Substrate Reagent. **Do not use a tablet that is not intact.**

Using clean pipettes and metal-free containers (such as plastic ware or acid-washed and distilled water-rinsed glassware) follow the procedure below:

1. Transfer 5 mL of Substrate Reagent for each tablet to be dissolved into a suitable container.
2. Transfer appropriate number of DAB Tablets (see DAB preparation chart) into measured amount of Substrate Reagent using non-metallic forceps or equivalent. Return desiccant to bottle immediately, if removed to obtain a tablet(s), and close bottle tightly. Allow tablet(s) to dissolve. The Chromogen-Substrate Solution must be used within 30 minutes.
3. Just prior to the dispensing for Color Development, swirl gently. While the presence of small particles will not interfere with assay results, the solution can be filtered without affecting staining intensity.

DAB Preparation Chart

No. of Slides	Tablets	Substrate Reagent
1–10	1	5 mL
11–20	2	10 mL
21–30	3	15 mL

STORAGE INSTRUCTIONS

1. Store kit reagents at 2 to 8°C.
2. Bring all kit reagents to room temperature for use and return to storage conditions indicated above immediately after use.

INDICATION OF INSTABILITY OR DETERIORATION OF REAGENTS

The Chromogen-Substrate Solution (DAB plus Substrate Reagent) should be colorless to pale reddish-brown. If the solution is dark brown in color, the solution has deteriorated and must be discarded.

QUALITY CONTROL

1. The Abbott PgR-ICA Control Slide (List 2A91) must be included with each assay run. Staining of the positive control with Primary Antibody should yield positive results. When the Abbott PgR-ICA Control Slide is used, greater than 20% cells stained indicates a valid assay. Note that the number, density, and staining intensity of cells may vary across different lots of Control Slides.

   NOTE: The Abbott PgR-ICA Control Slides confirm assay validity and are not quantitative controls.

2. A breast cancer tissue known to be negative by Abbott PgR-ICA Monoclonal may be included as a negative control.

   The frozen tissue sections should be prepared and stored as per instructions in "Preparation of Tissue Specimens".

3. Interpretation of results may be aided by staining an adjacent tissue section with hematoxylin and eosin (H and E).

SPECIMEN COLLECTION AND PREPARATION FOR ANALYSIS

NOTE: This procedure may be followed for preparation of control slides. See Quality Control Section for description of appropriate tissue to be used as controls. Specimens used for controls should have been stored continuously at –70°C or colder or in liquid nitrogen. Do not allow specimens to thaw at anytime. Alternatively, the Abbott PgR-ICA Control Slide may be used as a positive control. Include Control Slide at beginning of Step 6.

Use frozen or fresh specimens for this procedure. Routinely fixed paraffin-embedded specimens may produce inconsistent results.

A. Collection of tissue specimens.

1. Immediately place specimen in container on ice after excision.
2. As quickly as possible freeze the tissue at –70°C or colder in a cryostat or other rapid freezing device.

   NOTE: (1) Tissue may be stored for several months at –70°C or colder provided precautions are taken to prevent desiccation.

   (2) Failure to keep tissue cold or delay in freezing may result in loss of PgR.

B. Preparation of Tissue Specimens

NOTE: • Bring Methanol and Acetone to −10 to −25°C before use.
• Do not allow the tissue sections to dry at any time.

1. Cut two adjacent sections 4 to 6 microns thick from the tumor tissue. A third section may be cut if an H. & E section is to be prepared.
2. Place each section on a separate microscope slide that has been treated with the Tissue Adhesive. (See *Instructions for Preparation of Microscopic Slides.*)
   Caution: Do not allow the thaw-mounted sections to dry at anytime.
3. **Immediately** place the slides in 3.7% Formaldehyde-PBS for 10 to 15 minutes.
4. Transfer the slides to a slide rack in a PBS bath until the formaldehyde-PBS treatment is completed for all sections. The last slide should remain in the PBS bath for 4 to 6 minutes.
5. Using the slide rack, transfer the slides to cold methanol at −10 to −25°C for 3 to 5 minutes; then transfer to cold acetone at −10 to −25°C for 1 to 3 minutes. Rinse in a PBS bath at room temperature for 4 to 6 minutes.
6. Transfer the slides to a container of fresh PBS and rinse for an additional 4 to 6 minutes. If the Abbott PgR-ICA Control Slide is used, include it in this rinse.
7. Using a diamond-tip pen, make a circle around the tissue sections.
8. Return slides to a PBS bath.
9. Proceed to the *Immunocytochemical Staining Procedure* within 2 hours of completing fixation or, immediately after preparation, remove specimen from PBS and place in −10 to −20°C Specimen Storage Medium (see *Materials Required but not Provided*). Store at −10 to −20°C for up to four months.

PROCEDURE (See back page for ASSAY PROCEDURE)

Materials Provided

No. 2A08 ABBOTT PgR-ICA Monoclonal, 30 Test Units
(See REAGENTS for a Complete Listing)

Materials Required but not Provided

- Abbott PgR-ICA Control Slides (List 2A91) or equivalent positive control slide
- Absolute Methanol
- Acetone
- PBS — 0.01 *M* Phosphate Buffered Saline, pH 7.2 to 7.4: For each liter of PBS dissolve the following in 800 to 900 mL of distilled or deionized water:
  8.5 g Sodium Chloride
  1.43 g Potassium Phosphate (anhydrous), Dibasic (K_2HPO_4)
  0.25 g Potassium Phosphate (anhydrous), Monobasic (KH_2PO_4)
  Adjust final volume. Check pH and adjust if necessary. Store at 2 to 8°C. Make fresh weekly.
- 3.7% Formaldehyde-PBS Solution
  1 volume formaldehyde (37%)
  9 volumes PBS
  Make fresh daily.
  NOTE: This is the same as a 1:10 dilution of Formalin in PBS.
- Specimen Storage Medium
  For every 500 mL of Specimen Storage Medium, dissolve 42.8 g Sucrose and 0.70 g Magnesium Chloride (hexahydrate) in PBS.
  Adjust final volume to 250 mL.
  Add 250 mL glycerol and mix well by stirring.
  Store at −10 to −20°C.
- Sodium Hypochlorite Solution — 0.05%
  1 volume Clorox™ or equivalent
  100 volumes water
- Diluted Hematoxylin Counterstain
  1 volume 1% Harris Hematoxylin (such as Harleco)
  99 volumes distilled or deionized water
  Make fresh weekly.
- 95% Ethanol
- Absolute Ethanol
- Xylene
- Xylene or Toluene based mounting medium such as Permount, DPX or equivalent
- Dissecting knife
- Refrigeration unit maintained at −70°C or below or liquid nitrogen storage unit

- Cryostat
- Diamond-Tip Pen or Wax Pencil
- Staining Jars
- Slide Rack
- Microscope Slides
- Glass Cover Slips
- Timer
- Humidified Chamber
- Light Microscope equipped with Plan Achromat or equivalent lenses
- Precision Pipette to deliver Substrate Reagent
- Fume Hood
- Latex or Rubber Gloves
- Whatman No. 1 Filter Paper
- Filter Funnel
- Transfer Pipettes, plastic, disposable
- Freezer at −10 to −20°C
- Deionized or Distilled Water
- Tap Water

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INTERPRETATION OF RESULTS

Evaluation of this immunocytochemical technique should be performed by a specialist in breast cancer morphology and/or pathology.

Observations should be made using a bright-field microscope equipped with Plan Achromat, or equivalent, lenses. It is recommended that the visual evaluation of these stains be performed using Köhler illumination with a low voltage halogen illuminator, and that the final evaluation of percent cells stained be made using 250X magnification in order to maintain consistency in evaluation.

Illustrations of typical PgR-ICA staining patterns are shown in the Abbott PgR-ICA Monoclonal Staining Guide which is available through Abbott Laboratories Customer Support Center (1-800-323-9100).

Positive Control Slide Results

The immunocytochemical staining for progesterone receptor will be localized in the nuclei of the cells treated with the Primary Antibody. The staining should be reddish-brown and of various intensities. The nuclei of the cells treated with the Control Antibody should be blue due to the Hematoxylin counterstain. Presence of reddish-brown staining in the nuclei of the cells treated with the Primary Antibody indicates a valid assay.

Specimen Results

If progesterone receptor is present in the specimen, the immunocytochemical staining will be localized in the nuclei of the cells treated with the Primary Antibody and will appear reddish brown. The nuclei of the cells that do not contain a significant amount of progesterone receptor will appear as a low intensity blue stain. Other brown staining which might occur in cytoplasm, connective tissue, leukocytes, or erythrocytes or necrotic tissue will also be apparent in the specimen treated with Control Antibody and should be considered non-specific. Specimens with very high levels of PgR may have light brown cytoplasmic staining with the Primary Antibody.

The same specimen treated with Control Antibody, in place of the Primary Antibody, should not demonstrate significant nuclear staining. Staining observed in the Control Antibody section is considered non-specific.

If a negative control slide is run, no specific nuclear staining should be observed.

SPECIMEN EVALUATION

1. Determine the total percentage of the malignant cells that are non-specifically stained (brown nuclei) by scanning the entire section treated with the Control Antibody. Repeat this process to determine the total percentage of stained malignant cells on the section treated with the Primary Antibody. The percentage of malignant cells with detectable progesterone receptor is determined by subtracting the percentage of non-specifically stained malignant cells from the percentage of malignant cells stained by the Primary Antibody. Record the percentage of progesterone receptor-containing malignant cells. It is recommended that the percentage of cells stained be based upon counts of at least 200 tumor cells over a minimum of three representative microscopic fields.
2. Specimens with >10% malignant cells stained are considered PgR positive.
3. Specimens with ≤10% malignant cells stained are considered PgR negative.
4. Additional clinical and laboratory information should be considered in making individualized patient management decisions for specimens whose values are at or near the cutoff of 10% cells stained.
5. If moderate or strong nuclear staining is observed in any specimen treated with Control Antibody, there is reason to suspect that reagents were added incorrectly. The assay should be repeated.

LIMITATIONS OF THE PROCEDURE

Reproducible immunocytochemical staining of progesterone receptor is critically dependent upon exact adherence to the preceding directions.

Abbott PgR-ICA Monoclonal should be interpreted by a specialist in breast cancer morphology and/or pathology. Results should be used in conjunction with other clinical and laboratory data to make individualized patient management decisions.

The P_gR status of primary breast cancer lesions is useful in assessing patient prognosis following mastectomy. Abbott P_gR-ICA Monoclonal is not an absolute test for survival and disease-free interval. Each patient must be assessed on a case by case basis. While the result for each individual patient is not predictive, as a group patients with P_gR positive tumors have a longer time interval to recurrence and have longer overall survival than do patients with P_gR negative tumors.~~14-16~~ 13-15 P_gR-ICA positive patients exhibit longer overall survival and disease-free interval than P_gR-ICA negative patients, when patient groups are stratified by disease stage, menopausal status or pathological nodal status. (See *Expected Values*).

11
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CLINICAL TRIALS

~~EXPECTED VALUES~~

One hundred six breast tumor specimens from three institutions were assayed by the Abbott PgR-ICA Monoclonal and evaluated for the percentage of cells stained. Fifty-three specimens (50%) were classified as PgR-ICA positive (>10% cells stained). Fifty-three (50%) were classified as PgR-ICA negative (≤10% cells stained). Specimens included both primary and metastatic breast cancer lesions and were from patients with advanced breast cancer or primary breast cancer not surgically removed who were treated with hormonal therapy. Response to hormonal therapy was defined as objective regression ("responders"), progressive disease ("non-responders") or no change. Using a cutoff of 10% cells stained, a sensitivity of 69% [27 PgR-ICA(+) responders/39 total responders] and a specificity of 74% [37 PgR-ICA(-) non-responders/50 total non-responders] was obtained. See table below.

	Responders	Non-Responders	No Change
PgR-ICA(+)	27	13	13
PgR-ICA(-)	12	37	4

Sensitivity = 69%(27/39)
Specificity = 74%(37/50)

Fifty-one percent [27 PgR-ICA(+) Responders/53 Total PgR-ICA(+) patients] of the PgR-ICA(+) patients responded to hormonal therapy. Seventy-five percent [40 PgR-ICA(+) responders and no change patients/53 total PgR-ICA(+) patients] of the PgR-ICA(+) patients responded to or were stable on hormonal therapy. Seventy percent [37 PgR-ICA(-) non-responders/53 total PgR-ICA(-) patients] of the PgR-ICA(-) patients developed progressive disease on hormonal therapy.

A study of overall survival (time interval from mastectomy to death or date of last contact) included 325 primary breast tumor specimens. The survival of patients with PgR-ICA positive tumors (>10% cells stained) was significantly longer ($p<0.0001$) than that observed for patients with PgR-ICA negative (≤10% cells stained) tumors. PgR-ICA results were stratified by patient stage, menopausal status and pathologic nodal status. PgR positive patients in each category demonstrated statistically significantly longer overall survival than similarly stratified PgR negative patients ($p<0.05$). Survival rates at 36 and 60 months are shown in the following table:

	36 Months PgR-ICA(+)	36 Months PgR-ICA(-)	60 Months PgR-ICA(+)	60 Months PgR-ICA(-)
Total Population	96%	70%	90%	47%
Stage I	100%	86%	95%	67%
Stage II	94%	70%	87%	50%
Stage III	100%	57%	94%	19%
Premenopausal	96%	72%	84%	48%
Postmenopausal	96%	70%	92%	48%
0-3 Positive Nodes	98%	78%	94%	64%
>3 Positive Nodes	92%	60%	82%	31%

A similar study of disease-free interval (time interval from mastectomy to first breast cancer recurrence or, if no recurrence, date of last contact) included 322 of the 325 specimens evaluated for overall survival. Patients with PgR-ICA positive tumors (>10% cells stained) had significantly longer disease-free intervals than did patients with PgR-ICA negative (≤10% cells stained) tumors ($p<0.0001$). When PgR-ICA results were stratified by stage, menopausal status and pathologic nodal status, PgR positive patients demonstrated statistically significantly longer disease-free interval than PgR negative patients ($p<0.05$). Disease-free interval rates at 36 and 60 months are shown in the following table:

	36 Months PgR-ICA(+)	36 Months PgR-ICA(-)	60 Months PgR-ICA(+)	60 Months PgR-ICA(-)
Total Population	75%	40%	64%	27%
Stage I	95%	51%	77%	38%
Stage II	77%	40%	67%	29%
Stage III	40%	30%	30%	0%
Premenopausal	76%	38%	65%	20%
Postmenopausal	74%	40%	63%	28%
0-3 Positive Nodes	80%	50%	71%	39%
>3 Positive Nodes	68%	29%	50%	14%

SPECIFIC PERFORMANCE CHARACTERISTICS

Reproducibility

Assay reproducibility was determined by assaying tissue sections from four tumor specimens in replicates of three in four independent runs at four laboratories (n = 48). The Coefficient of Variation (% CV) was determined from the components of variance[22-24] and projected for assays of one tissue section per tumor. Results for the four tumors are presented in the following table:

Specimen	% Positive	Mean % Cells Stained	Within Run % CV	Between Run % CV
1	0(0/47)*	1	NA	NA
2	100(48/48)	44	18.0	21.2
3	100(48/48)	87	3.3	5.6
4	100(48/48)	93	2.0	3.0

*One slide at one site was not evaluated.
**NA = Not applicable.

The variance for Specimen 2 was related to an extremely heterogeneous and atypical breast cancer tumor. Using a cutoff of 10% cells stained, all investigators found Specimen 2 to be positive.

Specificity

Progesterone target and non-target tissues were assayed by PgR-ICA. Target tissues such as endometrium, uterine muscle, fallopian tube and human breast cancers known to respond to endocrine therapy exhibited PgR specific staining. Non-target tissues such as renal cell carcinoma, melanoma, liver carcinoma, sarcoma, laryngeal carcinoma, colon cancer, transitional cell carcinoma of the bladder, prostate adenocarcinoma, benign prostatic hypertrophy, normal liver and kidney did not exhibit PgR specific staining.

Tumor sections were incubated with potentially interfering substances prior to performing the PgR-ICA. The following were tested and did not show any interference in the assay:

Compound	Concentrations Tested
Aminoglutethimide	10^{-7} and 10^{-9}M
Androstan	10^{-7} and 10^{-9}M
Diethylstilbestrol	10^{-7} and 10^{-9}M
Dexamethasone	10^{-7} and 10^{-9}M
Estradiol	10^{-7} and 10^{-9}M
Megestrol Acetate	10^{-7} and 10^{-9}M
Prednisone	10^{-7} and 10^{-9}M
Progesterone	10^{-7} and 10^{-9}M
4-OH-Tamoxifen	10^{-7} and 10^{-9}M
Tamoxifen	10^{-7} and 10^{-9}M
Lidocaine	100 and 10 mg/mL

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IMMUNOCYTOCHEMICAL STAINING PROCEDURE

Preliminary Comments

NOTE: • Do not allow the slides to dry at any time during the staining procedure.
• Careful control of the timing of all steps is critical for reproducible results.

Prior To Performing The Assay:

1. Refer to *Specimen Collection and Preparation for Analysis.*
2. If fixed specimens which have been stored in Specimen Storage Medium are to be assayed, place them in a PBS bath for 5 minutes. Repeat using a fresh PBS bath. Proceed to Step 1 of the Assay Procedure.
3. Prepare a humidified chamber by placing moistened paper towels in a container with a lid. The chamber should contain a rack on which slides can be positioned horizontally.
4. Bring all reagents to room temperature for use.

Procedural Notes

1. Apply kit reagents dropwise in sufficient quantity to cover the circled tissue section; usually, 2 drops are sufficient.
2. Perform all incubation steps in the humidified chamber at room temperature.
3. Drain excess buffer or reagent from a slide onto a paper towel and then wipe the area around the etched circle with an absorbent wipe or equivalent.
   NOTE: Do not touch the tissue section on the slide. Touching will destroy the tissue.
4. If using the Abbott PgR-ICA Control Slides, do not allow the Primary Antibody and the Control Antibody to mix on the slide.
5. During the PBS washes following the incubation with PAP Complex, prepare the Chromogen Substrate Solution. Refer to *Instructions for Preparation of Chromogen Substrate Solution.*

Color Development

1. For dropwise addition of Chromogen Substrate Solution use either a disposable pipette or a pipette with a disposable tip. Rinse pipette or tip in Sodium Hypochlorite Solution for 15 minutes before disposal.
2. Incubation with Chromogen Substrate Solution must be carried out
3. Many disposal methods exist for DAB Chromogen Substrate Solution. Since disposal requirements may vary with different regions, local waste water authorities should determine the proper method of disposal in accordance with local regulations.

Counterstaining

If a precipitate appears in the diluted Hematoxylin Counterstain solution, the solution should be filtered through Whatman No.1 Filter Paper before use. Commercially available Harris Hematoxylin Solutions at 1% should be diluted 100 fold in distilled water prior to counterstaining the PgR slides. A very weak counterstaining solution is used to avoid masking the brown nuclear DAB staining.

Mounting

Change 95% Ethanol, Absolute Ethanol and Xylene weekly or every 100 slides to ensure complete dehydration of the tissue.

ASSAY PROCEDURE

See Preliminary Comments and Procedural Notes.

ABBOTT LABORATORIES
Diagnostics Division
North Chicago, IL 60064

1. Remove excess PBS from fixed specimen or control slide.
2. Add Blocking Reagent dropwise to each circle containing sample (specimen or control).
3. Incubate for 15 ± 2 minutes in humidified chamber.
4. Remove excess Blocking Reagent.
5. Add Primary Antibody dropwise to one slide of each specimen and control and add Control Antibody to a second slide from the same specimen and control. If using the Abbott PgR-ICA Control Slide, add the Primary Antibody to one circle on the slide and add Control Antibody to the other circle. Incubate 30 ± 2 minutes in humidified chamber.
6. Place slides in PBS bath for 5 ± 1 minutes. Repeat using fresh PBS.
7. Remove excess PBS.
8. Add Bridging Antibody dropwise to each sample. Incubate 30 ± 2 minutes in humidified chamber.
9. Place slides in a PBS bath for 5 ± 1 minutes. Repeat using fresh PBS.
10. Remove excess PBS.
11. Add PAP Complex dropwise to each sample. Incubate 30 ± 2 minutes in humidified chamber.
12. Place slides in a PBS bath for 5 ± 1 minutes. Repeat using fresh PBS.
13. Remove excess PBS.

COLOR DEVELOPMENT

14. Immediately add the Chromogen Substrate Solution dropwise to cover each sample. Incubate each sample 6 ± 1 minutes.
15. Drain excess Chromogen Substrate Solution from each slide into a beaker containing sodium hypochlorite solution (0.05% in tap water).
16. Immediately place slides in distilled or deionized water until all incubations with the Chromogen Substrate Solution are complete.
17. Rinse slides in gently running distilled or deionized water for 5 ± 1 minutes.

COUNTERSTAINING

18. Place the slides in a staining jar containing Diluted Hematoxylin Counterstain for 4 to 6 minutes.
19. Rinse slides in gently running tap water for 5 ± 1 minutes.

MOUNTING

20. Place the slides in a staining jar containing 95% ethanol for approximately 2 minutes. Repeat this step once using a second jar of 95% ethanol.
21. Place the slides in a staining jar containing absolute ethanol for approximately 2 minutes. Repeat this step once using a second jar of absolute ethanol.
22. Place the slides in a staining jar containing xylene for approximately 2 minutes. Repeat this step once using a second jar of xylene.
23. Add one drop of mounting medium such as Permount, DPX or equivalent to each coverslip.
24. Remove one slide at a time from xylene and immediately place coverslip over sample. Use two small coverslips for the Abbott PgR-ICA Control Slide. Wait a minimum of 10 minutes before viewing under the microscope.

DEPARTMENT OF HEALTH & HUMAN SERVICES — Public Health Service

P920006

Memorandum

Date SEP 15 1995

From Director, Office of Device Evaluation (HFZ-400)
Center for Devices and Radiological Health (CDRH)

Subject Premarket Approval of Diagnostic Products Corporation's Coat-A-Count® PSA IRMA - Action

To The Director, CDRH, ORA _____

ISSUE. Publication of a notice announcing approval of the subject PMA.

FACTS. Tab A contains a FEDERAL REGISTER notice announcing:

(1) a premarket approval order for the above referenced medical device (Tab B); and

(2) the availability of a summary of safety and effectiveness data for the device (Tab C).

RECOMMENDATION. I recommend that the notice be signed and published.

Susan Alpert, Ph.D., M.D.

Attachments
Tab A - Notice
Tab B - Order
Tab C - S & E Summary

DECISION

Approved _____ Disapproved _____ Date _____

Prepared by E. Radha, Ph.D., CDRH, HFZ-440, 9/11/95, 594-1293

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DEPARTMENT OF HEALTH AND HUMAN SERVICES

FOOD AND DRUG ADMINISTRATION

[DOCKET NO.]

Diagnostic Products Corporation.; Premarket Approval of Coat-A-Count® PSA IRMA

AGENCY: Food and Drug Administration, HHS.

ACTION: Notice.

SUMMARY: The Food and Drug Administration (FDA) is announcing its approval of the application by Diagnostic Products Corporation, Los Angeles, CA, for premarket approval, under section 515 of the Federal Food, Drug, and Cosmetic Act (the act), of Coat-A-Count® PSA IRMA. FDA's Center for Devices an Radiological Health (CDRH) notified the applicant, by letter on SEP 15 1995, of the approval of the application.

DATE: Petitions for administrative review by (insert date days after date of publication in the FEDERAL REGISTER).

ADDRESS: Written requests for copies of the summary of safety and effectiveness data, and petitions for administrative review to the Dockets Management Branch (HFA-305), Food and Drug Administration, Rm. 1-23, 12420 Parklawn Drive, Rockville, MD 20857.

FOR FURTHER INFORMATION CONTACT:

Peter E. Maxim, Ph.D.

Center for Devices and Radiological Health (HFZ -440)

Food and Drug Administration

2098 Gaither Road

Rockville, MD 20850

301-594-1294

SUPPLEMENTARY INFORMATION: On August 10, 1993, Diagnostic Products Corporation, Los Angeles, CA 90045, submitted to CDRH an application for premarket approval of Coat-A-Count® PSA IRMA. The device is an immunoradiometric assay intended for the quantitative measurement of prostate-specific antigen (PSA) in serum to aid in the management of prostate cancer patients. In accordance with the provisions of section 515(c)(2) of the act as amended by the Safe Medical Devices Act of 1990, this PMA was not referred to the Immunology Devices Panel, an FDA advisory panel, for review and recommendation because the information in the PMA substantially duplicates information previously reviewed by this panel.

On SEP 15, 1995, CDRH approved the application by a letter to the applicant from the Director of the Office of Device Evaluation, CDRH.

A summary of the safety and effectiveness data on which CDRH based its approval is on file in the Dockets Management Branch (address above) and is available from that office upon written request. Requests should be identified with the name of the device and the docket number found in brackets in the heading of this document.

OPPORTUNITY FOR ADMINISTRATIVE REVIEW

Section 515(d)(3) of the act (21 U.S.C. 360e(d)(3)) authorizes any interested person to petition, under section 515(g) of the act (21 U.S.C. 360e(g)), for administrative review of CDRH's decision to approve this application. A petitioner may request either a formal hearing under part 12 (21 CFR part 12) of FDA's administrative practices and regulations or a review of the application and CDRH's action by an independent advisory committee of experts. A petition is to be in the form of a petition for reconsideration under 10.33(b) (21 CFR 10.33(b)). A petitioner shall identify the form of review requested (hearing or independent advisory committee) and shall submit with the petition supporting data and information showing that there is

a genuine and substantial issue of material fact for resolution through administrative review. After reviewing the petition, FDA will decide whether to grant or deny the petition and will publish a notice of its decision in the FEDERAL REGISTER. If FDA grants the petition, the notice will state the issue to be reviewed, the form of the review to be used, the persons who may participate in the review, the time and place where the review will occur, and other details.

Petitioners may, at any time on or before (insert date 30 days after date of publication in the FEDERAL REGISTER), file with the Dockets Management Branch (address above) two copies of each petition and supporting data and information, identified with the name of the device and the docket number found in brackets in the heading of this document. Received petitions may be seen in the office above between 9 a.m. and 4 p.m., Monday through Friday.

This notice is issued under the Federal Food, Drug, and Cosmetic Act (secs. 515(d), 520(h), and (21 U.S.C. 360e(d), 360j(h)) and under authority delegated to the Commissioner of Food and Drugs (21 CFR 5.10) and redelegated to the Director, Center for Devices and Radiological Health (21 CFR 5.53).

Dated:_____.

_____

DEPARTMENT OF HEALTH & HUMAN SERVICES

Public Health Service

Food and Drug Administration
2098 Gaither Road
Rockville MD 20850

Edward M. Levine, Ph.D.
Manager, Clinical Affairs
Diagnostic Products Corporation
5700 West 96th Street
Los Angeles, California 90045

SEP 15 1995

Re: P930027
Coat-A-Count® PSA IRMA
Filed: August 10, 1993
Amended: January 21, 1994; April 8, 1994; August 3, 5, and 19, 1994; and September 11, 1995

Dear Dr. Levine:

The Center for Devices and Radiological Health (CDRH) of the Food and Drug Administration (FDA) has completed its review of your premarket approval application (PMA) for the Coat-A-Count® PSA IRMA. This device is an immunoradiometric assay indicated for the quantitative measurement of prostate-specific antigen (PSA) in serum to aid in the management of prostate cancer patients. We are pleased to inform you that the PMA is approved subject to the conditions described below and in the "Conditions of Approval" (enclosed). You may begin commercial distribution of the device upon receipt of this letter.

The sale, distribution and use of this device are restricted to prescription use in accordance with 21 CFR 801.109.

Expiration dating for this device has been established and approved at 60 days, stored at 2°C - 8°C. This is to advise you that the protocol you used to establish this expiration dating is considered an approved protocol for the purpose of extending the expiration dating as approved by 21 CFR 814.39 (a)(8).

CDRH will publish a notice of its decision to approve your PMA in the FEDERAL REGISTER. The notice will state that a summary of the safety and effectiveness data upon which the approval is based is available to the public upon request. Within 30 days of publication of the notice of approval in the FEDERAL REGISTER, any interested person may seek review of this decision by requesting an opportunity for administrative review, either through a hearing or review by an independent advisory committee, under section 515(g) of the Federal Food, Drug, and Cosmetic Act (the act).

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Failure to comply with the conditions of approval invalidates this approval order. Commercial distribution of a device that is not in compliance with these conditions is a violation of the act.

You are reminded that as soon as possible, and before commercial distribution of your device, that you must submit an amendment to this PMA submission with copies of all approved labeling in final printed form.

All required documents should be submitted in triplicate, unless otherwise specified, to the address below and should reference the above PMA number to facilitate processing.

PMA Document Mail Center (HFZ-401)
Center for Devices and Radiological Health
Food and Drug Administration
9200 Corporate Blvd.
Rockville, Maryland 20850

If you have any questions concerning this approval order, please contact Peter Maxim, Ph.D. at (301) 594-1293

Sincerely yours,

Susan Alpert, Ph.D., M.D.
Director
Office of Device Evaluation
Center for Devices and
Radiological Health

Enclosure

Summary of Safety and Effectiveness Data

Coat-A-Count® PSA IRMA

P930027

SUMMARY OF SAFETY AND EFFECTIVENESS DATA

I. GENERAL INFORMATION

Device Generic Name: Immunoradiometric test system for the quantitative measurement of prostate specific antigen (PSA) in human serum.

Device Trade Name: Coat-A-Count® PSA IRMA

Applicant's Name and Address: Diagnostic Products Corporation (DPC)
5700 West 96th Street
Los Angeles, California 90045

Premarket Approval Application (PMA) Number: P930027

Panel Recommendations: Pursuant to section 515(c)(2) of the act as amended by the Safe Medical Devices Act of 1990, this PMA was not the subject of an FDA Immunology Devices Advisory Panel meeting because the information in the PMA substantially duplicates information previously reviewed by this panel.

II. INDICATIONS FOR USE

Coat-A-Count® PSA IRMA is an immunoradiometric assay intended for the quantitative measurement of prostate-specific antigen (PSA) in serum to aid in the management of prostate cancer patients.

Background

Prostate specific antigen (PSA), first identified and characterized by Wang et. al, in 1979, is a glycoprotein monomer with protease activity.[1 2] PSA has an isoelectric point of approximately 6.9 and a molecular weight of approximately 33-34 kilodaltons, and contains approximately 10 percent carbohydrate. [1 2]

Amino acid sequencing [3 4] has been reported and the gene for PSA has been cloned.[4] PSA is biochemically and immunologically distinct from Prostatic Acid Phosphatase (PAP) and does not exhibit enzymatic phosphatase activity.[5] PSA is localized in the cytoplasm of prostatic ductal epithelium and in secretions of the ductal lumina.[6] Because PSA is a secretory protein of the prostate, it can be recovered and purified both from prostatic tissue and from seminal plasma.[7] PSA has been found to be exclusively associated with prostate tissue and elevated serum PSA has

B

been found in patients with prostate cancer, benign prostatic hypertrophy (BPH) or hyperplasia, and inflammatory conditions of other adjacent genitourinary tissues, but not in healthy men, men with nonprostatic carcinoma, healthy women or women with cancer.[5-8]

Serum PSA as measured with this device is not suitable as a screen for prostate cancer because elevated PSA concentrations are also observed in patients with BPH,[8] nor is it recommended as a guide in disease staging.

PSA determinations can be useful in detecting metastatic or persistent disease in patients following surgical or medical treatment of prostate cancer.[9,10] Persistent elevation of PSA following treatment or an increase in the pretreatment PSA concentration is indicative of recurrent or residual disease.[11-15] Therefore, PSA is accepted as an aid in the management of prostate cancer patients.[11-15] Concurrent measurement of PAP may contribute additional information.[16]

III. DEVICE DESCRIPTION

Diagnostic Products Corporation (DPC) Coat-A-Count® PSA IRMA herein referred to as the DPC PSA IRMA is an in vitro diagnostic medical device designed for the quantitative measurement of PSA in serum.

The patient sample or PSA calibrators and PSA Assay Buffer (PSAB) are added to PSA Antibody-Coated Tubes. PSA becomes bound to the surface of the tube. Iodine-125 labeled anti-PSA murine monoclonal antibody is added and PSA bound to the tube becomes labeled.

Unbound iodine-125 labeled anti-PSA is removed by washing. The tube is then placed in a gamma counter and the results are computed as percent of maximal binding. The PSA concentration is directly proportional to the radioactivity present in the tube. The concentration of PSA in the patient sample is obtained by interpolation from the calibration curve. The DPC PSA IRMA assay is calibrated to measure between 0 and 150 nanograms (ng) of PSA per milliliter (mL) of serum.

IV. ALTERNATIVE PRACTICES AND PROCEDURES

The DPC PSA IRMA is used in conjunction with routine medical practices and procedures in the management of prostate cancer patients. The following are alternative practices and procedures:

1) Serial determinations of prostatic acid phosphatase
2) Serial determinations of bone alkaline phosphatase

3) Serial determinations of total acid phosphatase
4) Serial determinations of total alkaline phosphatase
5) Bone scans
6) Whole body scans
7) Lymphangiography, lymphadenectomy, and biopsies
8) Ultrasonic and digital rectal examinations
9) Determination of PSA level/s with other legally marketed tests

V. MARKETING HISTORY

DPC PSA IRMA has been marketed in the following countries: Aubu Dhabi, Argentina, Boliva, Canada, PuertoRico, Haiti, Dominion Republic, Chile, Colombia, Costa Rica, Denmark, Greece, Gautemala, Netherlands, India, Italy, Jordan, Kuwait, Lebanon, Mexico, Panama, People's Republic of China, Peru, Philipines, Portugal, Saudi Arabia, Switzerland, Syria, Taiwan, Thailand, and Uruguay.

DPC PSA IRMA has not been withdrawn from the market in any country for any reason related to the safety and effectiveness of the device.

VI. Potential Adverse Effects of Device on Health

When the present device is used as indicated, and the results are evaluated in conjunction with all available clinical information, there are no known potential adverse effects to the health of patients undergoing management for cancer. False test results, though, could affect the physician's decisions regarding patient treatment. If falsely low, the physician may delay providing beneficial treatment in cases of recurring or progressive cancer. If falsely elevated, medical decisions may be made that result in needless therapy or change in treatment, including unnecessary surgical or radiation procedures.

Precautions

The DPC PSA IRMA assay is not approved as a screening test or to be used as a sole diagnostic tool. PSA levels should not be used as absolute evidence of presence or absence of malignant disease.

The concentrations of PSA in a given specimen determined with different assays can vary due to differences in assay methods and reagent specificity. The results reported by the laboratory to the physician must include the identity of the assay used. Values obtained with PSA assays from different manufacturers cannot be used interchangeably. Before changing assays, the laboratory must confirm baseline values for patients being serially monitored.

Since elevated levels of PSA have been reported in some patients with non-malignant diseases of the prostate, such as BPH, this device alone should not be used to screen for or diagnose prostate cancer.

Warnings

PSA expression may be altered due to hormonal therapy for prostate cancer. Consequently, a low PSA result following a prostatic cancer treatment which includes hormonal therapy may not adequately reflect the presence of residual or recurrent disease.[17]

Specimens from patients who have received preparations of mouse monoclonal antibodies for diagnosis or therapy may contain human anti-mouse antibodies (HAMA) and may result in erroneous results.

Manipulations of the prostate (e.g. biopsy, transurethral resection and prostatectomy) can lead to transient and even high increases in circulating PSA.[18] Studies on whether digital rectal examination affects PSA levels have had conflicting results.[19 20] Blood samples should be obtained prior to any manipulation of the prostate whenever possible. If this is not possible, it is important to note the exact time of sampling for PSA analysis in relation to any prior manipulation of the prostate. PSA has an estimated circulating half-life of 2.2 days. Serum samples should be obtained approximately 3 weeks after manipulation of the prostate to avoid any effect on the PSA level due to manipulation.

A single PSA value should not be the basis for decisions regarding patient care. Repeat determinations utilizing serially drawn specimens are recommended.

The PSA normal range is not applicable when managing patients who have been treated or are currently receiving treatment for their disease. In patients who have undergone surgery for complete removal of the prostate, the presence of any detectable PSA indicates the possible presence of residual prostate tissue, possibly of a cancerous nature, and should be investigated.[12]

VII. SUMMARY OF STUDIES

Preclinical laboratory studies, assay performance studies, clinical studies, and clinical utility studies were conducted.

A. Preclinical studies

Preclinical laboratory studies were conducted to determine the identity and specificity of the reagents.

1. Characterization of the antigen

Purity and identity of the PSA used to immunize goats and in the affinity purification of the goat polyclonal antibody were characterized by SDS polyacrylamide gel electrophoresis and amino acid analysis. The results from these studies showed agreement with values previously reported in the literature for purified PSA.[1 - 4]

2. Characterization of Antibodies

The DPC PSA IRMA anti-PSA monoclonal(mouse) antibody was characterized with respect to subclass type and affinity constant. Specificity of the anti-PSA monoclonal antibody was demonstrated by the presence of a single band in a Western blot. Specificity of DPC PSA IRMA polyclonal (goat) antibody was also demonstrated. These studies confirmed the specificity of the antibody pair used in the DPC PSA IRMA. Interferences from substances commonly found in patient sera, from chemotherapeutic drugs and from other drugs potentially used in the prostatic cancer patients were evaluated. No cross reactivity was observed at any concentration spanning the anticipated levels of alpha fetoprotein, carcinoembryonic antigen, ferritin, prolactin, triglycerides, or total human serum protein. No crossreactivity was observed at any concentration spanning the anticipated serum levels of cyclophosphamide, diethylstilbestrol, doxorubicin, methotrexate, megesterol, flutamide, lupon, estramustine, hydroxyflutamide, or at high concentrations of 24 other non-chemotherapeutic drugs.

B. Assay Performance

Assay performance studies were conducted to determine the performance characteristics of the assay.

1. Reproducibility

Within run (intra-assay), between run (inter-assay), between laboratory, and between lot reproducibility was evaluated in a series of assay

runs of the DPC PSA IRMA at Diagnostic Products Corporation and three outside sites using the same three lots of reagents. Samples prepared by enriching serum with various amounts of PSA were analyzed by the DPC PSA IRMA. Each specimen was analyzed in replicates of six per assay. Assays at each of the four sites were repeated five times with each of three lots for a total of 60 assays. The coefficient of variation (CV) for intra-assay, inter-assay, inter-laboratory site, and inter-lot sources of variation is given at four different clinically important PSA levels:

Mean intra-assay CV's

5% at approximately 2 ng/mL (range 4 to 6%);
3% at approximately 5 ng/mL (range 2 to 4%);
3% at approximately 10 ng/mL (range 2 to 4%);
3% at approximately 25 ng/mL (range 2 to 4%)

Mean inter-assay CV's

7% at approximately 2 ng/mL (range 4 to 12%);
5% at approximately 5 ng/mL (range 1 to 8%);
4% at approximately 10 ng/mL (range 3 to 7%);
5% at approximately 25 ng/mL (range 2 to 9%)

Mean inter-laboratory site CV's

5% at approximately 2 ng/mL (range 3 to 6%);
4% at approximately 5 ng/mL (range 4 to 5%);
6% at approximately 10 ng/mL (range 5 to 8%);
5% at approximately 25 ng/mL (range 4 to 5%)

Mean inter-lot CV's

2.5% at approximately 2 ng/mL (range 1 to 5%);
3% at approximately 5 ng/mL (range 1 to 4%);
4% at approximately 10 ng/mL (range 2 to 5%);
3% at approximately 25 ng/mL (range 2 to 3%)

In addition, 81 DPC PSA IRMA assays were conducted on combinations of three lots of antibody coated tubes, labeled antibody, calibrators, and assay buffer. CV's were calculated for the PSA values obtained for controls assayed in all 81 assays. These CV's represented all sources of variability combined, including within-assay, between assay, and between-lot. Coeffients of variation were less than 10% for analyte levels of 2-80 ng/mL. These CVs are acceptable for an assay of this type.

2. Analytical Sensitivity

The sensitivity of the DPC PSA IRMA is calculated to be approximately 0.1 ng/mL. This concentration is defined as the concentration at two standard deviations above the DPC PSA IRMA "A" or "Zero" Calibrator and represents the lowest measurable concentration of PSA that can be distinguished from zero, also noted as the detection limit or minimal detectable dose.

3. Parallelism

Multiple dilution of each of 21 specimens from prostate cancer patients containing elevated PSA concentrations were assayed by the DPC PSA IRMA undiluted and diluted with the zero calibrator. Linear regression analysis of PSA concentration as a function of dilution yielded correlation coefficients of 0.9986 or better for all specimen. The mean observed/expected value for the dilutions was 106 percent. The coefficient of variation for the PSA concentrations calculated from the dilutions yielded a mean of 4.1 percent, and ranged from 2 to 7 percent.

4. Recovery

Varying concentrations of PSA were added to eight prostate cancer serum samples. The endogenous and spiked concentrations of PSA in each sample were assayed using the DPC PSA IRMA. The percent recoveries were calculated and found to range from 88-100 percent with a mean recovery of 96 percent.

5. Potential interference

Spiking recovery interference studies revealed that neither icterus (20 mg/dL bilirubin) nor hemolysis (30 uL hemolyzed packed red cells) had any clinically significant effect on the DPC PSA IRMA assay.

6. Method Comparison Study

Correlation and linear regression analyses were applied to serum PSA measurements obtained by the DPC PSA IRMA assay and the comparison immunoassay for which an approved premarket application for the measurement of PSA exists. Test results of 2700 serum specimens, with PSA levels ranging from 0 to approximately 6000 ng/mL, were analyzed using

linear regression. The resulting correlation coefficient was 0.987, the slope was 1.035, and the intercept was - 1.627 ng/mL.

7. Stability

PSA Calibrators, PSA Assay Buffer, PSA Ab-Coated Tubes, Buffered Wash Solution, and Iodine-125 labeled anti-PSA murine monoclonal antibody were subjected to stability studies. These included elevated temperatures for varying numbers of days, conditions recommended for use in the package insert for varying numbers of days, and under conditions used in-house for long term storage for varying numbers of days. Following the indicated conditions, the components were tested in an assembled kit format using the DPC PSA IRMA assay.

No deterioration was observed in the reagents under the conditions of the stability studies. The data support one year shelf-life for calibrators, assay buffers, Ab-coated tubes, buffered wash solution; 60 days of shelf-life for I 125 labeled anti-PSA monoclonal antibody and 60 days for the DPC PSA IRMA assay stored at 2-8° C.

Serum samples should be stored at 2-8° C if they are to be assayed within 24 hours. If samples are to be assayed after extended storage, samples should be stored at -20° C.

C. Clinical Studies

Clinical studies were performed at three medical institutions to determine PSA values using DPC PSA IRMA assay and to demonstrate comparability of the method of quantitation to another device for which there is an approved PMA.

The three investigators who conducted these studies were: Lynn Witherspoon, M.D., Ochsner Clinic, New Orleans, LA; Robert Vessella, Ph.D., University of Washington, Department of Urology, Seattle, WA; and Herbert A. Fritsche, Ph.D., M.D., Anderson Cancer Center, Division of Laboratory Medicine, University of Texas, Houston, TX.

Serum specimens (2710) were obtained from a total of 1578 subjects and patients consisting of 470 healthy male subjects with negative digital rectal exams, 204 female subjects, 363 male patients with nonmalignant diseases, 131 patients with non-prostatic malignancies,

and 410 patients with prostatic malignancies. Serial serum specimens were obtained from 166 patients with malignancies (161 prostatic cancer; 5 colon cancer). The distribution of the PSA values in these 1578 individuals is shown in TABLE 1. The nonmalignant conditions included BPH, prostatitis, and benign diseases of the colon, bladder, liver, pancreas, testes, lung, kidney, and central nervous system. The malignant conditions included testicular, pancreatic, bladder, colon, kidney, liver, pulmonary, renal, pancreatic, rectal, and stomach cancers.

The distribution of serum PSA levels from healthy individuals and patients with nonmalignant and malignant disease are presented in TABLE 1. The distribution of serum PSA values using the DPC PSA IRMA in healthy subjects and patients with nonmalignant and malignant disease matched closely the distribution of PSA values using the comparison method (98.6 percent).

A summary of the correlation between the DPC PSA IRMA and the comparison method is presented in TABLE 2. The number of specimens listed represents the number of specimens available for testing by both the DPC PSA IRMA and comparison method which were included in the analysis.

TABLE 1

Distribution of PSA by DPC PSA IRMA
All Investigational Sites

		PSA(ng/mL)				
Patient Category		0.00-4.00	4.01-10.00	10.01-20.00	20.01-40.00	> 40.00
Healthy Male Subjects	470	468	2	0	0	0
Female Subjects	204	204	0	0	0	0
Non-malignant Diseases	363	305	44	10	2	2
Non-Prostatic Malignancies	131	115	12	1	1	2
Prostate Cancer	410	247	63	29	23	48
Total	1578	1339	121	40	26	52

For serially monitored patients, this table includes only the 1st available specimen.

Table 2
Summary of Correlation studies

Specimen Type	N	Corr. Coeff.	Slope	Y-Intercept
Healthy Male Subjects	470	0.93	0.942	0.018
BPH Patients	308	0.99	0.996	-0.032
Nonprostate Cancer Patients	124	0.99	1.012	-0.017
Prostate Cancer Patients	1518	0.99	1.036	-2.843

D. Clinical Utility as Demonstrted by Serial Samples

Clinical studies were performed to evaluate DPC PSA IRMA to aid in the management of patients diagnosed with prostate cancer.

Serial samples from 161 patients clinically diagnosed with prostate cancer were assayed retrospectively by both the DPC PSA IRMA and a comparison method for which there is an approved PMA. Monitoring studies included 106 patients from New Orleans, 25 patients from University of Washington, and 30 patients from M.D.Anderson Cancer Center.
Of the 161 patients with prostate cancer, 36 patients initially presented at diagnosis with stage A disease, 29 with stage B disease, 22 patients with stage C disease, and 65 patients with stage D disease. One patient was diagnosed with oat cell carcinoma and there was no stage information for eight patients.

Serial measurement of PSA concentrations using both the DPC PSA IRMA and the comparison method reflected the progression or remission of the disease in 145 of the 152 cases (95.4 percent) which could be classified into one of 6 clinical groups. The nine cases which could not be classified into one of the six clinical groups nevertheless showed the same trends over time with both methods.

The results of the studies are summarized as follows:

1. 19 patients' serum PSA concentrations decreased following effective therapy,

2. 49 patients' serum PSA concentrations remained in the normal reference range in the absence of active or progressive disease,

3. 29 patients' serum PSA concentrations were or became elevated above the normal reference range in the presence of active or progressive disease,

4. 48 patients' serum PSA concentrations paralleled the clinical course of the disease with periods of disease progression and periods of response to therapy,

5. 5 patients' serum PSA concentrations were elevated above the normal reference range in the absence of clinically detectable disease or while the patient was in remission, and

6. 2 patients' serum PSA concentrations were in the normal reference range in the presence of clinically progressive disease.

VIII. Conclusions drawn from the studies

The foregoing studies have demonstrated the safety and effectiveness of DPC PSA IRMA to determine the concentration of PSA in human serum to aid in the management of prostate cancer patients. Based upon the results of preclinical studies, assay performance (reproducibility, analytical sensitivity, parallelism, recovery, potential interference, method comparison, and stability), clinical correlation, and clinical utility studies cited above, the DPC PSA IRMA performance specifications are within acceptable limits for a device of this type.

The distribution of PSA concentrations as determined by the DPC PSA IRMA demonstrates agreement with the values obtained with a device for which there is an approved PMA.

The data from the serially monitored prostate cancer patients support the clinical utility of the DPC PSA IRMA as a test to aid in the management of prostate cancer.

Based on the data provided in the PMA, CDRH has concluded that the device is reasonably safe and effective for the stated indications.

IX. Panel Recommendation

Pursuant to section 515(c)(2) of the act as amended by the Safe Medical Devices Act of 1990, this PMA was not referred to the Immunology Devices Panel for review and recommendation because the information in the PMA substantially duplicates information previously reviewed by this panel.

B

X. CDRH Action on the application

CDRH issued an approval order for applicant's PMA for Coat-A Count® PSA IRMA to Diagnostic Products Corporation on _____.

The applicant's manufacturing facilities were inspected on May 5, 1995 and the facilities were found to be in compliance with the Good Manufacturing Practice (GMP) Regulations.

The shelf-life of the DPC PSA IRMA and I 125 labeled anti-PSA monoclonal antibody has been established at 60 days and one year for calibrators, assay buffers, Ab-coated tubes and buffered wash solution when stored at 2°- 8°C.

XI. Approval Specifications

Direction for Use: See attached labeling (Attachment A).

Conditions of approval: CDRH approval of this PMA is subject to full compliance with the conditions described in the approval order (Attachment B).

XII. References

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COAT-A-COUNT®

PSA IRMA

Caution: Federal law restricts this device to sale by or on the order of a physician.

DPC

Coat-A-Count® PSA IRMA

Intended Use

Coat-A-Count PSA IRMA is an immunoradiometric assay intended for the quantitative measurement of prostate-specific antigen (PSA) in serum to aid in the management of prostate cancer patients.

Catalog numbers: **IKPS1** (100 tubes), **IKPS2** (200 tubes)

The 100-tube kit contains not more than 20 microcuries (740 kilobecquerels) of radioactive ^{125}I monoclonal anti-PSA; the 200-tube kit contains not more than 40 microcuries (1480 kilobecquerels).

The concentration of PSA in a given specimen determined with assays from different manufacturers can vary due to differences in assay methods and reagent specificity. The results reported by the laboratory to the physician must include the identity of the assay used. Values obtained with different PSA assays cannot be used interchangeably. Before changing assays, the laboratory must confirm the baseline values for patients being serially monitored.

Summary and Explanation of the Test

Prostate specific antigen (PSA), first identified and characterized by Wang et al in 1979, is a glycoprotein monomer with protease activity.[1,2] PSA has an isoelectric point of approximately 6.9 and a molecular weight of approximately 33-34 kilodaltons, containing approximately 10% carbohydrate by weight.[1,2] Subsequently, the amino acid sequence of PSA was reported,[3] and the gene has been cloned.[4] PSA is biochemically and immunologically distinct from PAP and does not exhibit enzymatic phosphatase activity.[5]

PSA is localized in the cytoplasm of prostatic ductal epithelium and in secretions of the ductal lumina.[6] Because PSA is a secretory protein of the prostate, it can be recovered and purified both from prostatic tissue and from seminal plasma.[7] PSA has been found to be exclusively associated with prostate tissue, and elevated serum PSA has been found in patients with prostate cancer, benign prostatic hypertrophy, and inflammatory conditions of other adjacent genitourinary tissues, but not in healthy men, men with nonprostatic carcinoma, healthy women or women with cancer.[5,8]

Serum PSA is not suitable as a screen for prostate cancer because elevated PSA concentrations are also observed in patients with benign prostatic hypertrophy.[8]

PSA determinations can be useful in detecting metastatic or persistent disease in patients following surgical or medical treatment of prostate cancer.[9,10] Persistent elevation of PSA following treatment or an increase in the pretreatment PSA concentration is indicative of recurrent or residual disease.[11-15] Hence, PSA is widely accepted as an aid in the management of prostate cancer patients.[11-15] Concurrent measurement of PAP may contribute additional information.[16]

Principle of the Procedure

Coat-A-Count PSA IRMA is a solid-phase immunoradiometric assay based on monoclonal and polyclonal anti-PSA antibodies: one ^{125}I-labeled anti-PSA monoclonal antibody in liquid phase, and one polyclonal anti-PSA antibody immobilized to the wall of a polystyrene tube.

- PSA is captured between polyclonal anti-PSA antibodies immobilized on the inside surface of the polystyrene tube and the radio-labeled monoclonal anti-PSA tracer.
- Unbound ^{125}I-labeled anti-PSA antibody is removed by decanting the reaction mixture and washing the tube; this reduces nonspecific binding to a very low level, and ensures low-end precision.
- The PSA concentration is directly proportional to the radioactivity present in the tube after the wash step. The radioactivity is counted using a gamma counter, after which the concentration of PSA in the patient sample is obtained by comparing the patient counts-per-minute with those obtained for the set of calibrators provided.

Procedure

There are only two reagents to dispense, and total incubation time is 1 hour. The tracer has a high specific activity, with total counts of approximately 300,000 cpm at iodination. No centrifuge is required. The Coat-A-Count procedure is suitable for high-volume testing.

Separation

The coated-tube methodology offers significant advantages in reliability, as well as speed and convenience, since the tubes can be vigorously decanted without loss of antibody-bound material. This results in a clean separation of bound from free, with negligible nonspecific binding.

Calibration

The assay has a calibration range of 1.5 to 150 ng/mL.

Precision

CVs are low and uniform. The assay can detect as little as 0.1 ng/mL, and no "end-of-run" effect has been observed in assays involving up to 200 tubes.

Accuracy

Extensive experiments have shown that the assay is accurate over a broad range of PSA values. Its accuracy has been further verified in a patient comparison study against a commercially available immunoradiometric assay for PSA.

Specificity

The kit is specific for PSA, with low crossreactivity to other proteins and polypeptides present in patient samples.

Warnings

- For *in vitro* diagnostic use.
- Some individuals have antibodies to mouse protein which can cause interference in immunoassays that employ antibodies derived from mice. Specimens from patients who have received preparations of mouse monoclonal antibodies for diagnosis or therapy, in particular, may contain human anti-mouse antibodies (HAMA). These specimens may show erroneous results in such assays.[20-22] Therefore, Coat-A-Count PSA IRMA results should be used only in conjunction with results from some other diagnostic procedure and information available from the clinical evaluation of the patient.

Precaution

- Before opening the kit, review the paragraphs on safety printed on the inside front cover, as they relate to the safe handling and disposal of reagents containing radioactivity, human serum and sodium azide.

Materials Supplied: Initial Preparation

PSA Ab-Coated Tubes (IPS1)

100 (200*) polystyrene tubes coated with goat polyclonal antibodies to PSA and packaged in zip-lock bags. Store refrigerated and protected from moisture, carefully resealing the bags after opening: stable at 2–8°C until the expiration date marked on the bag.

^{125}I PSA Ab (IPS2)

Two vials (four vials*) of lyophilized reagent, consisting of an iodinated anti-PSA murine monoclonal antibody, with preservative. Reconstitute each vial by adding a measured **5.5 mL** distilled water. Let stand for 10 minutes, then mix by gentle inversion. Store refrigerated: stable at 2–8°C for at least 30 days after reconstitution, or until the expiration date marked on the label.

PSA Calibrators (PSI3–9)

One set of seven vials, labeled A through G, of PSA calibrators in a non-human serum/buffer matrix, with preservative. The calibrators are supplied in liquid form, ready to use. The zero calibrator A contains 3 mL, while the remaining calibrators B through G each contain 1 mL. Store refrigerated: stable at 2–8°C for at least 30 days after opening. The life of the calibrators may be extended by freezing. Aliquot if necessary to avoid repeated freezing and thawing.

The calibrators contain, respectively, 0, 1.5, 3, 10, 50, 100 and 150 nanograms of PSA per milliliter (ng/mL). Intermediate calibration points may be obtained by mixing calibrators in suitable proportions.

PSA Assay Buffer (PSAB)

One vial (two vials*) containing 11 mL of buffered diluent with preservative. Store refrigerated: stable at 2–8°C for at least 30 days after opening, or until the expiration date marked on the vial.

Buffered Wash Solution Concentrate (2PSBW)

One vial (two vials*) each containing 60 mL of a concentrated buffered saline solution, with surfactants and sodium azide as a preservative. Using a transfer container, dilute each vial of concentrate with **600 mL** distilled water, for a total volume of 660 mL. Store refrigerated: stable at 2–8°C for at least 6 months after preparation.

* 200-tube kit.

Materials Required But Not Provided

- Gamma counter – compatible with standard 12×75 mm tubes
- Rack shaker – set at approximately 200 strokes per minute. Available from DPC as catalog numbers DPSR1 (110 VAC) and DPSR2 (220 VAC).

Reagent Preparation

- Distilled or deionized water
- Pipet – to deliver 5.5 mL
- Graduated cylinder – for dispensing 600 mL
- Plastic storage container with lid – for preparation and storage of Buffered Wash Solution

Immunoassay

- Micropipets: 50 µL and 100 µL. For the 100 µL reagent additions, a reliable repeating dispenser (Nichiryo Model 8100 or equivalent) is recommended – available from DPC.
- Dispenser – for delivering 2.0 mL of Buffered Wash Solution. A 2.0 mL dispenser is available from DPC as catalog number DB2ML.
- Foam decanting rack – available from DPC.

Specimen Collection

The patient need not be fasting, and no special preparations are necessary. Collect blood by venipuncture into plain tubes, taking care to avoid hemolysis, and separate the serum from the cells. Note the time of collection. The procedure calls for 50 µL per tube.

Neither bilirubin nor hemolysis interferes with the assay.

Samples should be obtained before biopsy, prostatectomy or prostatic massage, since manipulation of the prostate gland may lead to elevated PSA levels persisting for up to 3 weeks.[17] Studies have shown conflicting results on the existence of an effect of digital rectal examination on PSA level.[18,19] Therefore, when possible, obtain PSA samples prior to digital rectal examination.

Store serum samples at 2–8°C if they are to be assayed within 24 hours. Store at –20°C or colder if samples are to be assayed after extended storage. Before assay, allow the samples to come to room temperature and mix by *gentle* swirling or inversion. Aliquot, if necessary, to avoid repeated thawing and freezing. Do *not* attempt to thaw frozen specimens by heating them in a waterbath. Dilute patient samples with high concentrations in the zero calibrator before assay, so that the response falls within the calibration range of the assay.

mmunometric Assay Procedure

All components must be at room temperature (18°C – 27°C) before use.

1 Label fourteen PSA Ab-Coated Tubes A (nonspecific binding) and B through G ("maximum binding") in duplicate. Label additional antibody-coated tubes, also in duplicate, for controls and patient samples.

Calibrators	ng/mL
T*	—
A (NSB)	0
B	1.5
C	3
D	10
E	50
F	100
G(MB)	150

* Optional

2 Pipet **50** µL of each calibrator, control and patient serum sample into the tubes prepared.

Pipet directly to the bottom. *Patient samples expected to contain high concentrations should be diluted in the zero calibrator before assay. The use of disposable-tip micropipets is recommended, to avoid carryover from sample to sample. Positive-displacement pipets and automatic pipettor-diluters should be used only if the possibility of carryover has been evaluated and found to be insignificant.*

3 Add **100** µL of PSA Assay Buffer to all tubes (except T).

Pipet directly to the bottom. *Make sure that sample and buffer are in solution and thoroughly mixed, without foaming. A repeating dispenser (Nichiryo or equivalent) is recommended.*

4 Shake for **30 minutes** on a rack shaker.

5 Decant thoroughly. Add **2 mL** Buffered Wash Solution to each tube. Wait 1 to 2 minutes, then decant thoroughly.

Removing all visible moisture will greatly enhance precision. Using a foam decanting rack, decant the contents of all tubes (except the T tubes) and allow them to drain for 2 or 3 minutes. Then strike the tubes sharply on absorbant paper to shake off all residual droplets.

6 Add **100** µL of ^{125}I PSA Ab to every tube.

Set the (optional) T tubes aside for counting (at step 9); they require no futher processing.

7 Shake for **30 minutes** on a rack shaker.

8 Decant thoroughly. Add **2 mL** Buffered Wash Solution to each tube. Wait 1 to 2 minutes, then decant thoroughly. Again add **2 mL** Buffered Wash Solution, wait 1 to 2 minutes, and decant thoroughly.

Removing all visible moisture will greatly enhance precision. Using a foam decanting rack, decant the contents of all tubes and allow them to drain for 2 or 3 minutes. Then strike the tubes sharply on absorbant paper to shake off all residual droplets.

9 Count for **1 minute** in a gamma counter.

In multi-head gamma counters, the (optional) Total Counts tubes should be separated from the remaining assay tubes by at least one space, to minimize the possibility of spillover.

Calculation and Quality Control

To calculate PSA concentrations from a log-log representation of the calibration curve, first correct the counts per minute (CPM) of each pair of tubes by subtracting the average CPM of the nonspecific binding tubes (calibrator A):

Net Counts = Average Counts *minus* Average **NSB** Counts

Then determine the binding (%B/B_{150}, here called "%B/MB") of each pair of tubes as a percent of maximum binding, with the NSB-corrected counts of the highest calibrator (calibrator G) taken as 100%:

Percent Bound = (Net Counts / Net **MB** Counts) × 100

Using the 3-cycle log-log graph paper supplied with the kit, plot Percent Bound versus Concentration for each of the nonzero calibrators, and draw a curve approximating the path of these points. (Connect the calibration points with arcs or straight line segments. Do not attempt to fit a single straight line to the data.) PSA concentrations for controls and unknowns within range of the nonzero calibrators may then be estimated from the calibration curve by interpolation. An additional plot of Percent Bound versus Concentration for the three lowest calibrators on linear-linear graph paper may be used for interpolation near zero dose.

Comments: Although other approaches are acceptable, data reduction by the method just described has certain advantages from the standpoint of quality control. In particular, it yields a calibration curve that is *relatively* linear in both log-log and linear-linear representations, and relatively stable from assay to assay. It also yields valuable QC parameters, namely, Percent Bound (%B/B_{150} or "%B/MB") values for the nonzero calibrators.

A still more informative graph, conveying a sense of within-assay reproducibility as a function of concentration, can be obtained by plotting the Percent Bound values of individual calibrator tubes directly, i.e. without first averaging the CPM of replicates.

Alternatives: Although Percent Bound can be calculated directly from Average CPM, correction for nonspecific binding usually produces a calibration curve that is more nearly linear throughout its range. A calibration curve can also be constructed by plotting CPM or Average CPM directly against Concentration on either log-log or linear-linear graph paper. (Semi-log graph paper should *not* be used.) This approach has the virtue of simplicity, but is less desirable from the standpoint of quality control.

Computerized Data Reduction: "Point-to-point" methods, including linear and cubic spline fits, are suitable for use with the Coat-A-Count PSA IRMA system. However, since they provide little assistance in monitoring the integrity of an assay, it is important to prepare the recommended log-log plot of the calibration curve, either manually or by computer, as a quality control step.

Data reduction techniques based on the logistic model may also be applicable. Within this family, curve-fitting routines based on the 4- or 5-parameter logistic are the most suitable candidates. Bear in mind, however, that some algorithms currently in use may not converge successfully, even when the logistic model is true to the data. If a logistic method is adopted, it is essential to verify its appropriateness for each day's assay by monitoring the backcalculation of the calibrators, and other parameters. In addition, a plot of the calibration curve in a log-log representation is highly recommended, as this is more informative than the conventional semi-log plot.

Sample Handling: The instructions for handling and storing patient samples and components should be carefully observed. Dilute patient samples with high concentrations in the zero calibrator before assay. All samples, including the calibrators and controls, should be assayed in duplicate. It is important to use a *disposable-tip* micropipet, changing the tip between samples, to avoid carryover contamination. Positive-displacement pipets and automatic pipettor-diluters should be used only if the possibility of carryover has been evaluated and found to be insignificant. Pairs of control tubes may be spaced throughout the assay to help verify the absence of significant drift. Inspect the results for agreement within tube pairs, and take care to avoid carryover from sample to sample.

Gamma Counter: To minimize the possibility of spillover in multi-well gamma counters, the (optional) total counts tubes (T) should be separated by one or more spaces from the other assay tubes. Alternatively, add only 25 µL of the ^{125}I PSA Ab to each of the T (total counts) tubes at step 6, and multiply the observed counts per minute in these tubes by 4.

Controls: Controls or serum pools with at least two PSA concentration levels (low and high) should routinely be assayed as unknowns, and the results charted from day to day as described in Westgard JO, et al. A multi-rule chart for quality control. Clin Chem 1981;27:493-501. Repeat samples are a valuable additional tool for monitoring interassay precision.

QC Parameters: We recommend keeping track of these performance measures:

T = Total Counts (as counts per minute)

%NSB = 100 × (Average NSB Counts / Total Counts)

%MB = 100 × (Net MB Counts / Total Counts)

And the Percent Bound (%B/B_{150} or "%B/MB") values of all but the highest of the nonzero calibrators, for example:

%C/MB = 100 × (Net Calibrator "C" Counts) / (Net MB Counts).

Record Keeping: It is good laboratory practice to record for each assay the lot numbers and reconstitution dates of the components used, as well as control results and QC parameters.

Further Reading: A technical bulletin titled "Coat-A-Count TSH IRMA: Notes on Data Reduction, QC and Optimization" (catalog number: ZJ019) is available on request. See Dudley RA, et al. Guidelines for immunoassay data reduction. Clin Chem 1985;31:1264-71.

Example

The values below are intended for illustration only and should not be used to calculate results from another assay.

Tube	Duplicate CPM	Average CPM	Net CPM	Percent Bound	PSA ng/mL
T	261,413 259,305	260,359			
A (NSB)	287 208	256	0		0
B	1,677 1,588	1,633	1,377	1.5%	1.5
C	2,865 2,814	2,840	2,584	2.9%	3
D	9,128 8,848	8,988	8,732	9.7%	10
E	40,028 39,017	39,523	39,267	44%	50
F	67,069 66,631	66,850	66,594	74%	100
G ("MB")	90,231 88,810	89,521	89,265	100%	150
Unknowns					
X1	2,103 2,086	2,095	1,839	2.0%	2.1
X2	4,203 4,151	4,177	3,921	4.4%	4.5
X3	18,678 18,075	18,377	18,121	20%	22

Quality Control Parameters: T = 260,359 cpm
%NSB = 0.10%
%MB = 35%

Performance Data

In the sections below, PSA results are expressed as nanograms of PSA per milliliter (ng/mL).

Precision

The reliability of DPC's Coat-A-Count PSA IRMA procedure was assessed by examining its reproducibility on samples selected to represent a range of PSA levels.

Intraassay (Within-Run): Statistics were calculated for each of three samples from the results of 20 pairs of tubes in a single assay. Results are expressed in ng/mL.

Sample	Mean	SD	CV
1	2.3	0.10	4.3%
2	4.5	0.24	5.3%
3	24	0.83	3.5%

Interassay (Run-to-Run): Statistics were calculated for each of three samples from the results of pairs of tubes in 20 different assays. Results are expressed in ng/mL.

Sample	Mean	SD	CV
1	2.3	0.09	3.9%
2	4.6	0.20	4.3%
3	24	0.92	3.8%

Sensitivity

The assay's detection limit, defined as the concentration two standard deviations above the response at zero dose, is approximately 0.1 ng/mL.

Drift

To determine whether there is any position (or "end-of-run") effect due to delays in the addition of reagents, pairs of tubes were spaced throughout a long assay for each of six samples. The results show no significant position effect even in assays of up to 200 tubes.

Sample	Tubes 17–28	Tubes 69–80	Tubes 121–132	Tubes 187–198
1	2.1	2.2	2.3	2.2
2	2.8	2.9	3.0	2.8
3	4.3	4.6	4.5	4.5
4	20	20	21	21
5	23	23	23	24
6	41	41	42	42

Specificity

The specificity of the CAC PSA IRMA was analyzed by testing sera containing the compounds tabulated below. These compounds did not show interference, either positive or negative, at the levels indicated.

Compound	Amount Added
Interfering substances:	
Albumin	9 g/dL
Alpha-fetoprotein (AFP)	10,000 ng/mL
Carcinoembryonic antigen (CEA)	10,000 ng/mL
Ferritin	10,000 ng/mL
Human chorionic gonadotropin (HCG)	10,000 mIU/mL
Human IgG	1,600 mg/dL
Prostatic acid phosphatase (PAP)	1,000 ng/mL
Prolactin	2,000 ng/mL
Triglycerides	2,000 mg/dL
Protein	16.5 g/dL
Chemotherapeutic agents:	
Cyclophosphamide	1,000 µg/mL
Diethylstibesterol	10,000 ng/mL
Doxorubicin HCL	100 µg/mL
Methotrexate	100 µg/mL
Megesterol acetate	1,000 µg/mL
Flutamide	100 µg/mL
Lupron	100 µg/mL
Estramustine	1,000 µg/mL
Hydroxyflutamide	100 µg/mL

In addition, the specificity of the Coat-A-Count PSA IRMA was also analyzed by testing sera containing the compounds listed below. These compounds did not show interference, either positive or negative, at the level of 100,000 ng/mL.

Acetaminophen	Furosemide
Acetylsalicyclic acid	Gentamicin
Albuterol	Hydrochlorothiazide
Alprazolam	Hydromorphone
Aminophylline	Ibuprofen
Amitryptylline	Indomethacin
Ascorbic acid	Metaproterenol
Atropine	Morphine
Caffeine	Phenobarbital
Clorpropamide	Phenylpropanolamine
Codeine	Secobarbital
Diazepam	Theophylline

Parallelism

Four patient serum samples were assayed both undiluted and diluted with the zero calibrator. The observed and expected values are presented below in ng/mL.

Sample	Dilution	O Observed	E Expected	%O/E
1	20 in 20	50	—	—
	10 in 20	26	25	104%
	4 in 20	10	10	100%
	2 in 20	5.3	5.0	106%
	1 in 20	2.7	2.5	108%
2	20 in 20	66	—	—
	10 in 20	33	33	100%
	4 in 20	14	13	108%
	2 in 20	7.1	6.6	108%
	1 in 20	3.6	3.3	109%
3	20 in 20	80	—	—
	10 in 20	40	40	100%
	4 in 20	16	16	100%
	2 in 20	8.3	8.0	104%
	1 in 20	4.2	4.0	105%
4	20 in 20	126	—	—
	10 in 20	65	63	103%
	4 in 20	27	25	108%
	2 in 20	14	13	108%
	1 in 20	7.0	6.3	111%

Spiking Recovery

Three spiking solutions were prepared using the zero calibrator as diluent. The solutions (A, B and C) were made to represent 60, 261 and 575 ng/mL, respectively. A 50 µL aliquot of each solution was spiked into 950 µL aliquots of four different patient serum samples, for a spiking ratio of 1 to 19, leaving the serum matrix of the spiked samples relatively intact. All samples were then assayed by the Coat-A-Count PSA IRMA procedure. To calculate expected values, 95% of the unspiked value was added to 5% of the spiking solution concentration (3.0, 13 and 29 ng/mL, respectively).

Sample	Spiking Solution	O Observed	E Expected	% O/E
1	—	1.5	—	—
	A	4.3	4.4	98%
	B	14	14	100%
	C	29	30	97%
2	—	42	—	—
	A	42	43	98%
	B	51	53	96%
	C	63	69	91%
3	—	86	—	—
	A	81	85	95%
	B	90	95	95%
	C	109	110	99%
4	—	106	—	—
	A	100	104	96%
	B	109	114	96%
	C	123	129	95%

Method Comparison

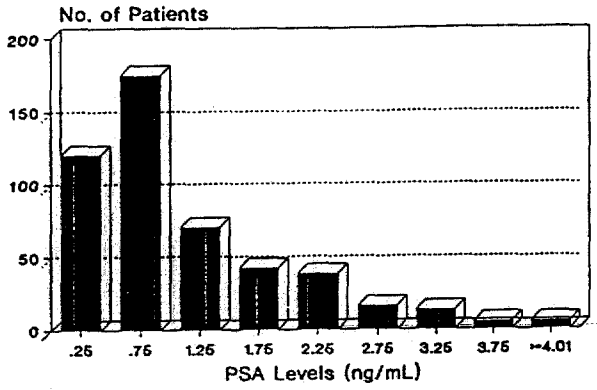
A method comparison study was conducted at three clinical sites involving 2,604 specimens with PSA concentrations within the calibration range of the Coat-A-Count PSA IRMA and the reference assay. Subjects included prostatic cancer patients (single specimens and serial specimens); patients with benign prostatic disease, other nonmalignant diseases, nonprostatic cancers; and individuals with normal digital rectal exams. 204 female subjects were also included. Linear regression analysis yielded the following statistics.

(CAC IRMA) = 0.99 (Reference Assay) + 0.171 ng/mL $r = 0.987$

Mean: 5.4 ng/mL (CAC IRMA)
5.3 ng/mL (Reference Assay)

Expected Values

PSA values were studied in 470 healthy male subjects with normal digital rectal exams studied at three clinical sites. Approximately 97% of the subjects were over 40 years of age. The distribution of PSA values measured by DPC's Coat-A-Count PSA IRMA is shown below. 468 out of 470 subjects (99.5%) had PSA values of less than 4 ng/mL. Based upon this distribution, an upper limit of normal of 4 ng/mL was established.



Laboratories should consider the reference range limit suggested by this study *as a guideline only*. Because of differences which may exist between laboratories and locales with respect to population, laboratory technique and selection of reference groups, it is important for each laboratory to establish by similar means the appropriateness of adopting the reference range suggested here.

Limitations

1 Serum PSA concentrations should not be interpreted as absolute evidence for the presence or absence of malignant disease, nor should serum PSA be used as a screening test for malignant disease.[8]

2 Prediction of malignant prostatic disease recurrence should be based on a complete clinical evaluation of the patient, which may also include serial serum PSA determinations.

3 Samples should be obtained before biopsy, prostatectomy or prostatic massage, since manipulation of the prostate gland may lead to elevated PSA levels persisting up to 3 weeks.[17]

4 To evaluate the "high-dose hook" effect characteristic of immunoradiometric assays, samples containing PSA values up to 39,000 ng/mL were assayed by the Coat-A-Count PSA IRMA procedure and found to yield results well above 150 ng/mL, the concentration of the highest calibrator.

5 PSA expression may be altered due to hormonal therapy for prostate cancer. Consequently, a low PSA result following a prostatic cancer treatment which includes hormonal therapy may not adequately reflect the presence of residual or recurrent disease.[23]

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Technical Assistance

For questions regarding the Coat-A-Count PSA IRMA kit or its reagents, or for further advice on technique, data reduction, quality control or expected values, please contact DPC's Technical Services department or your National Distributor.

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